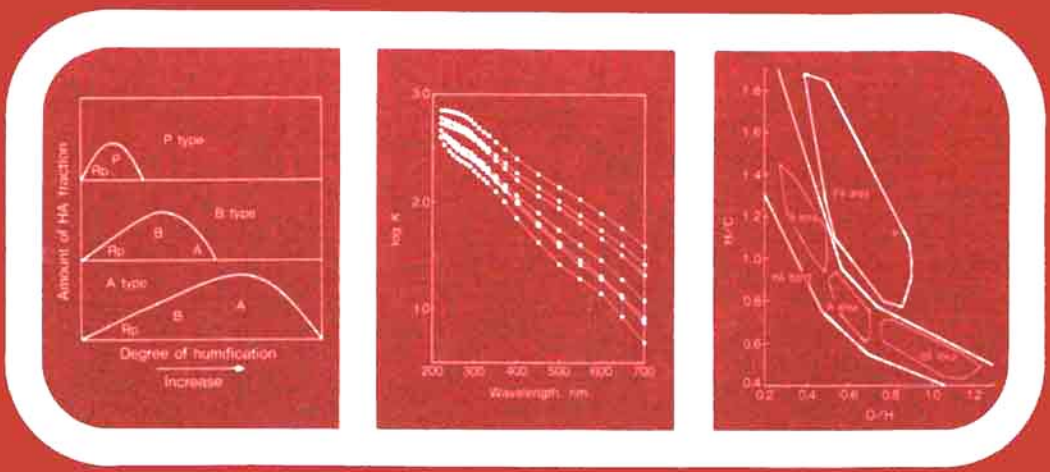
KYOICHI KUMADA

CHEMISTRY OF SOIL ORGANIC MATTER



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CHEMISTRY OF SOIL ORGANIC MATTER

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JAPAN SCIENTIFIC SOCIETIES PRESS Tokyo
ELSEVIER Amsterdam—Oxford—New York—Tokyo
1987

Copublished by
JAPAN SCIENTIFIC SOCIETIES PRESS, Tokyo

and
ELSEVIER SCIENCE PUBLISHERS, Amsterdam

exclusive sales rights in Japan
JAPAN SCIENTIFIC SOCIETIES PRESS
6-2-10 Hongo, Bunkyo-ku, Tokyo 113

for the U.S.A. and Canada
ELSEVIER SCIENCE PUBLISHING COMPANY, INC.
52 Vanderbilt Avenue, New York, NY 10017

for the rest of the world
ELSEVIER SCIENCE PUBLISHERS
25 Sara Burgerhartstraat
P.O.Box 211, 1000 AE Amsterdam, The Netherlands

ISBN 0-444-98936-6 (Vol. 17)
ISBN 0-444-40882-7 (Series)

ISBN 4-7622-0534-6 (Japan)

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Supported in part by The Ministry of Education, Science and Culture under Grant-in-Aid for Publication of Scientific Research Result.

Printed in Japan

Dedicated to my teacher
Professor Kenzo Kobo
and
to the memory of another teacher
Professor Matsusaburo Shioiri
and
my good friend
Dr. Martyn Hurst

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Preface

The author began to study humus shortly after the end of World War II, and continued until 1984 when he retired from Nagoya University. He has compiled in this book facts and a discussion of soil organic matter (humus) based on his experimental results during the past 40 years.

Throughout his study, the author had the intention of constructing a "shrine"—the establishment of a systematic understanding of soil organic matter. A shrine built on a small island in the Far East might appear too insignificant to attract the interest of the rest of the world. Although the shrine is still under construction and has many deficiencies of design and fabrication, it is the author's duty and responsibility as designer and supervisor to record all the many aspects involved. Because specificity connotes universality, he does not feel anxious about the strangeness of the structure, but wonders whether his description is adequate for reader understanding.

The chief god of the shrine is humic acid. Fulvic acid is a lesser god, and humin is found at a corner of the altar. The current popularity of the cult of humic substances would suggest that these should have been the chief god, but the author dared instead to choose humic acid for the reasons given in Chapter 1.

While the shrine has been constructed for the pleasure of the accomplishment itself, at the same time, it will hopefully contribute to a clarification of the role of organic matter in soil formation and an understanding of pedogenesis in terms of humus chemistry. Elucidation of the role of soil organic matter in crop production has not been included here, because it is considered that under the extremely industrialized agriculture in Japan organic matter such as crop residues, farmyard manure, compost, and city organic waste are more important in maintaining and improving the fertility of arable soils.

Despite the large number of papers and books published on soil organic matter, our knowledge of the subject is still very limited. In this context, Kononova (1975) stated: "Although many questions concerning the nature and properties of soil organic matter remain obscure, this is not due to lack

of energy on the part of research workers, but rather to the complexity of the problem." The author would like to add that the complexity of the problem is nothing but the complexity of soil organic matter *per se*, which has retarded the development of a sound methodology.

The author's personal opinion is that our knowledge of even humic acid, the most closely studied fraction of humus, remains at the stage of prescience; one reason is that we have not yet succeeded in drawing its chemical configuration. This volume contains one opinion on what is needed in order for humus chemistry to grow into a true science.

The ultimate mission is to answer the question of what humus is. But most knowledge of humus is concentrated on humic acid, very little on fulvic acid and humin. Descriptions herein are therefore limited primarily to humic acid.

An understanding of humic acid, one of the most of nature's elements requires its classification, as has been done for plants, animals, and minerals. If the classification is reasonable, it can be used as the basis for elucidation of the diversity of any properties of humic acid, and may also explain the genesis of humic acid and its genetic relations.

The author classified humic acid into several types and studied its nature and properties. A novel method for humus composition analysis was proposed and applied to various kinds of soils in Japan and several other countries. The treatises presented here are based on these experimental results. Topics are restricted to those with universal validity.

The author's greatest pleasure will be realized if this book can represent a tangible portion of the shrine he has long envisioned to advance progress in humus science.

Kyoichi Kumada

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Chapter 1

Introduction

1.1. Usage of Terms

Discussions regarding terminology in the field of humus at the 8th International Congress of Soil Science (ICSS) meeting in Bucharest in 1964, indicated clearly the diversity of opinions regarding the terms of soil organic matter (SOM) and their usage. No further discussions have been held on this matter and no unified opinion yet seems to have been reached.

The statements of Hayes and Swift (1978) based on the grouping of SOM proposed by Kononova (1966, 1975) are quoted here, and the author's own terminology will be explained.

Hayes and Swift statements: "The complete soil organic fraction is made up of live organisms and their undecomposed, partly decomposed and completely transformed remains. SOM is the term used to refer more specifically to the non-living components which are a heterogeneous mixture composed largely of products resulting from microbial and chemical transformations of organic debris. These transformations, known collectively as the humification process, give rise to humus, a mixture of substances which have a degree of resistance to further microbial attack.

"Since SOM is a heterogeneous mixture, it is necessary for purposes of discussion to subdivide it into groups which have similar morphological or chemical characteristics. Here we are adopting a classification based largely on proposals by Kononova (1966, 1975). In this system SOM is separated into two major groups:

"(I) Unaltered materials, which include fresh debris and non-transformed components of older debris;

"(II) Transformed products, or humus, bearing no morphological resemblance to the structures from which they were derived. These transformed components are often referred to as humified products, but in fact they consist of both humic and non-humic substances and can be therefore subdivided as follows:

"(IIa) Amorphous, polymeric, brown-colored humic substances which are differentiated on the basis of solubility properties into humic acids (HAs), fulvic acids (FAs) and humins.

"(IIb) Compounds belonging to recognizable classes, such as polysaccharides, polypeptides, altered lignins, *etc.*. These can be synthesized by microorganisms or can arise from modifications of similar compounds in the original debris."

Comments

(1) In the grouping system cited above, SOM is separated into two major groups: (I) unaltered materials and (II) transformed products, or humus. While this grouping is basically understandable, such a division is practically impossible because the boundary between the two groups is obscure, and we have neither practical means nor criteria for separating them.

Humus is subdivided into humic and non-humic substances, and this is also generally understandable. But from the standpoint of experimental science, such a categorization is almost meaningless for the reason mentioned.

(2) The popularity of the cult of humic substances was noted in the Preface and in this context, some comments on the term "humic substances" are added.

Hayes and Swift (1978) stated that humic substances are amorphous, polymeric, brown-colored matter whose structures and properties were little understood. It is true that we know little about the chemical configurations of humic substances; they are at present "unknown and undefinable" substances in terms of chemistry. On the other hand, non-humic substances belong to known and definable classes of organic chemistry.

It is impossible to distinguish unknown substances from known substances; this is a matter of logic. When we speak of the separation of humic and non-humic substances, it is assumed that they are a mixture of separable constituents. Is this premise true?

(3) Humus is extracted from soil by a suitable extractant and is divided into HA and FA (see (5) below). The HA and FA are often called humic substances despite there being no procedures for removing non-humic substances. This is not correct usage according to the classification by Kononova. Even if a treatment to remove non-humic substances from HA and FA is adopted, the treated HA and FA cannot be called "pure" humic substances, because of the reason mentioned above.

(4) Excerpts from speeches delivered by Hayes and Russell at the 1964 discussion include: "I would propose that in order to define humus, we must refer to it as a soluble material (Hayes)." "Any definitions we have must be definitions which have worked within the laboratory. In practice, we cannot define, unless we can measure what we define (Russell)."

(5) In this book, SOM and humus are used synonymously. Extractable humus, that is, humus extracted from soil with 0.1 N NaOH, 0.1 M $Na_4P_2O_7$, or their mixture, is divided into acid-precipitable and non-precipitable fractions, respectively called HA and FA. It is assumed that both are made up of humic and non-humic substances.

(6) Throughout this book, a clearer definition of the concepts of the terms in humus chemistry is sought, especially humic substances and humification.

The terms humic substances and humification are also widely used in the fields of sediment and water. It is therefore, interesting and important from a chemical standpoint to compare the concepts of these terms as they are used in each field.

1.2. Brief Explanation of the Soils of Japan

A large majority of the soils dealt with in this book were sampled in Japan, and most of them were forest soils, although Alpine grassland soils, Alpine meadow soils, and some Black soils existed under grassland. Agricultural soils were, in principle, excluded, because they are reclaimed and their humus composition has been somewhat modified by agricultural practices. Peat soils were also excluded because they are hydromorphic and not formed under terrestrial conditions.

For reader edification, the classification system of forest soils in Japan is explained briefly and the classification of alpine soils by Ohsumi (1969) and Ohsumi and Kumada (1971) will also be shown.

Foreign soils were collected in Great Britain, Czechoslovakia, Canada, Thailand, and Nepal and their classification primarily based on that of the respective country.

Classification of forest soil

Based on the soil classification system proposed by Ohmasa (1951), a new system of classifying forest soil was set up by the Forest Soils Division of the Japanese Government Forest Experiment Station in 1975. This classification system (the FES System) is adopted here.

As seen in Table 1-1, forest soils are divided into 8 soil groups. The soil group corresponds to Bodentyp of West Germany and Marbuts' great

TABLE 1-1
Classification of forest soils in Japan (1975) (Forest Soils Division, 1976)

Soil Group	Sub-group	Type	Subtype	
Podzolic soils				P
	Dry podzolic soils			P_D
		Dry podzol		P_{DI}
		Dry podzolic soil		P_{DII}
		Dry slightly podzolic soil		P_{DIII}
	Wet iron podzolic soils			$P_W(i)$
		Wet iron podzol		$P_W(i)_I$
		Wet iron podzolic soil		$P_W(i)_{II}$
		Wet iron slightly podzolic soil		$P_W(i)_{III}$
	Wet humus podzolic soils			$P_W(h)$
		Wet humus podzol		$P_W(h)_I$
		Wet humus podzolic soil		$P_W(h)_{II}$
		Wet humus slightly podzolic soil		$P_W(h)_{III}$
Brown forest soils				B
	Brown forest soils			B
		Dry brown forest soil (loose granular structure type)		B_A
		Dry brown forest soil (granular and nutty structure type)		B_B
		Weakly dried brown forest soil		B_C
		Moderately moist brown forest soil		B_D
			Moderately moist brown forest soil (drier subtype)	$B_D(d)$
		Slightly wetted brown forest soil		B_E
		Wet brown forest soil		B_F
	Dark brown forest soils			dB
		Moderately moist dark brown forest soil		dB_D
			Moderately moist dark brown forest soil (drier subtype)	$dB_D(d)$
		Slightly wetted dark brown forest soil		dB_E
	Reddish brown forest soils			rB
		Dry reddish brown forest soil (loose granular structure type)		rB_A
		Dry reddish brown forest soil (granular and nutty structure type)		rB_B
		Weakly dried reddish brown forest soil		rB_C
		Moderately moist reddish brown forest soil		rB_D
			Moderately moist reddish brown forest soil (drier subtype)	$rB_D(d)$
	Yellowish brown forest soils			yB
		Dry yellowish brown forest soil (loose granular structure type)		yB_A
		Dry yellowish brown forest soil (granular and nutty structure type)		yB_B
		Weakly dried yellowish brown forest soil		yB_C
		Moderately moist yellowish brown forest soil		yB_D
			Moderately moist yellowish brown forest soil (drier subtype)	$yB_D(d)$
		Slightly wetted yellowish brown forest soil		yB_E
	Surface gleyed brown forest soils			gB
		Dry surface gleyed brown forest soil (granular and nutty structure type)		gB_B

TABLE 1-1—Continued

Soil Group	Sub-group	Type	Subtype
			Weakly dried surface gleyed brown forest soil ..... *g*B$_C$
			Moderately moist surface gleyed brown forest soil ..... *g*B$_D$
			Slightly wetted surface gleyed brown forest soil ..... *g*B$_E$
Red and Yellow soils ..... RY			
	Red soils ..... R		
			Dry red soil (loose granular structure type) ..... R$_A$
			Dry red soil (granular and nutty structure type) ..... R$_B$
			Weakly dried red soil ..... R$_C$
			Moderately moist red soil ..... R$_D$
			Moderately moist red soil (drier subtype) ..... R$_D$(d)
	Yellow soils ..... Y		
			Dry yellow soil (loose granular structure type) ..... Y$_A$
			Dry yellow soil (granular and nutty structure type) ..... Y$_B$
			Weakly dried yellow soil ..... Y$_C$
			Moderately moist yellow soil ..... Y$_D$
			Slightly wetted yellow soil ..... Y$_E$
	Surface gleyed red and yellow soils ..... *g*RY		
			Strongly surface gleyed red and yellow soil ..... *g*RY$_I$
			Weakly surface gleyed red and yellow soil ..... *g*RY$_{II}$
			Strongly bleached red and yellow soil ..... *g*RYb$_I$
			Weakly bleached red and yellow soil ..... *g*RYb$_{II}$
Black soils ..... B*l*			
	Black soils ..... B*l*		
			Dry black soil (granular and nutty structure type) ..... B*l*$_B$
			Weakly dried black soil ..... B*l*$_C$
			Moderately moist black soil ..... B*l*$_D$
			Moderately moist black soil (drier subtype) ..... B*l*$_D$(d)
			Slightly wetted black soil ..... B*l*$_E$
			Wet black soil ..... B*l*$_F$
	Light colored black soils ..... *l*B*l*		
			Dry light colored black soil (granular and nutty structure type) .... *l*B*l*$_B$
			Weakly dried light colored black soil ..... *l*B*l*$_C$
			Moderately moist light colored black soil ..... *l*B*l*$_D$
			Moderately moist light colored black soil (drier subtype) *l*B*l*$_D$(d)
			Slightly wetted light colored black soil ..... *l*B*l*$_E$
			Wet light colored black soil ..... *l*B*l*$_F$
Dark red soils ..... DR			
	Eutric dark red soils ..... *e*DR		
			Dry eutric dark red soil (loose granular structure type) ..... *e*DR$_A$
			Dry eutric dark red soil (granular and nutty structure type) ..... *e*DR$_B$
			Weakly dried eutric dark red soil ..... *e*DR$_C$
			Moderately moist eutric dark red soil ..... *e*DR$_D$
			Moderately moist eutric dark red soil (drier subtype) ..*e*DR$_D$(d)
			Slightly wetted eutric dark red soil ..... *e*DR$_E$
	Dystric dark red soils ..... *d*DR		

TABLE 1-1—Continued

Soil Group	Sub-group	Type	Subtype	
			Dry dystric dark red soil (loose granular structure type)	dDR$_A$
			Dry dystric dark red soil (granular and nutty structure type)	dDR$_B$
			Weakly dried dystric dark red soil	dDR$_C$
			Moderately moist dystric dark red soil	dDR$_D$
			Moderately moist dystric dark red soil (drier subtype)	dDR$_D$(d)
			Slightly wetted dystric dark red soil	dDR$_E$
		Volcanogenous dark red soils		vDR
			Dry volcanogenous dark red soil (loose granular structure type)	vDR$_A$
			Dry volcanogenous dark red soil (granular and nutty structure type)	vDR$_B$
			Weakly dried volcanogenous dark red soil	vDR$_C$
			Moderately moist volcanogenous dark red soil	vDR$_D$
			Moderately moist volcanogenous dark red soil (drier subtype)	vDR$_D$(d)
			Slightly wetted volcanogenous dark red soil	vDR$_E$
Gley soils				G
	Gley			G
		Gley		G
	Pseudogley			psG
		Pseudogley		psG
	Podzolic gley			PG
		Podzolic gley		PG
Peaty soils				Pt
	Peat soil			Pt
		Peat soil		Pt
	Muck soil			Mc
		Muck soil		Mc
Immature soils				Im
	Immature soil			Im
		Immature soil		Im
	Eroded soil			Er
		Eroded soil		Er

soil group in the USA. Subgroup and soil type correspond approximately to Subtyp and Variatät of West Germany, respectively. The explanations of Podzol, Brown forest soil, Red and Yellow soil, and Black soil which are dealt with here are cited from the English summary of the paper presented by the Forest Soils Division (1975).

1. Podzol

The soils of the Podzol group have well developed A_0, eluvial, and illuvial horizons with strong acidity. In general, these soils develop in cool and moist regions in Japan. The Podzols are subdivided into three subgroups, Dry podzol, Wet iron podzol, and Wet humus podzol.

Dry podzols are distributed mainly in subalpine and alpine zones, but can also be found in mountainous regions of temperate zones. They are distributed at relatively dry sites such as mountain tops, ridges, upper convex slopes, or the rims of plateaus. In addition to these topographic features, acidic parent material, a sandy texture, and certain vegetation such as *Thujopsis dolabrata* or *Sciadopitys verticillata* accelerate the podzolization. The horizon sequence is well developed A_0(F), A or H-A, A_2(eluvial), B_1(illuvial), and B_2 horizon.

Soils of the Wet iron podzol subgroup are formed from the clayey and compact parent material at a gentle ridge, peneplain, or plateau of volcanogenous mud flow. The soils are distributed in temperate or subalpine zones under vegetation of natural forests of *Picea glehnii, Abies mariessi, Pinus parviflora, Thuja standishii, Chamaecyparis obtusa,* or *Fagus crenata.*

Although Wet iron podzol is classified as a member of the Podzol group, the surface gleyzation, which is indicated by high ferrous iron content in the A_0 and A_1 horizons, is considered to be a major characteristic of this subgroup. The horizon sequence is well developed A_0(H), A or H-A, A_2-g, B_1 or B_1-g, and B_2 horizon. The soils have a massive structure, and some have an iron pan in the upper B horizon.

Soils of Wet humus podzol are strongly influenced by surface gleyzation and have high ferrous iron content in the H and A horizons. The sola of the soils are not so compact as those of Wet iron podzols, so that humus penetrates more deeply. These soils have a thick greasy H horizon, a thick A horizon which is rich in humus, and a darker B horizon. They have a dark grey portion in the A horizon accompanied by a dark rust-colored iron-rich portion underneath.

Soils of Wet humus podzol are distributed in upper temperate and subalpine zones under the vegetation of forests of *Picea mariesii, Abies veigchii, Picea hondoensis, Chamaecyparis obtusa, Thuja standishii, Betula ermanii,* or *Fagus crenata.*

2. Brown forest soil

The soils of the Brown forest soil group have a horizon sequence of (A_0)-A-B-C, and have no eluvial or illuvial horizon, a brown-colored B horizon, and slight or strong acidity.

The Brown forest soils are distributed in rather wider ranges of temperate and warm temperate zones in humid (high precipitation) climates. The soils are zonal, formed between the zones of Podzols and Red and Yellow soils. They have a wide variety of characteristics or indications of maturity and some are influenced by the soil formation process of other soil groups. Consequently, soils in this group are classified into five subgroups: typical Brown forest soil; Dark brown forest soil which is distributed close to the

Podzol zone and has features similar to Wet humus podzol; Reddish brown forest soil and Yellowish brown forest soil which are accompanied and influenced by the Red and Yellow soils; and Surface gleyed brown forest soil which is influenced by surface gleyzation.

3. Red and Yellow soil

The concept widely accepted for the genesis of Red and Yellow soils is that they are zonal soils formed in subtropical regions under a moist climatic condition. However, the results of recent pedological surveys and research have shown Red soils in Japan to be relic soils formed during a warm period of the geological era. The Yellow soils are distributed in the same areas and often accompany the Red soils. Some of these have yellow topsoil and red subsoil in a profile, and others have yellowish orange color reflecting the red-colored weathered material in yellow-colored sola.

Although Red soils and Yellow soils are supposed to have a close relationship, this relationship and the difference of the genesis of the two soils have not yet been clarified. In Okinawa, the southernmost and most subtropical region of Japan, the Yellow soils are commonly found in the mountains, and are considered by some to be formed in those climatic conditions. Therefore, Yellow soil may be considered as an independent soil group in the future.

The soils of this group are subdivided into three subgroups by soil color and surface gleyzation.

4. Black soil

The soils of the Black soil group (Ando soil, Kurobokudo; Wada, 1986) have thick black or brownish-black A horizon. The boundary of the A and B horizons is distinct. Their bulk density is low, while their water-holding capacity and base exchange capacity are high. The Black soils are separated into two subgroups according to the degree of blackness of the A horizon.

The Black soils are distributed mainly on grasslands and are rarely found in areas thought to have long been covered by forests. On the other hand, the black color of the topsoil of the Black soil in grasslands fades after the repeated plantation of forest trees. These facts suggest that grassland vegetation is a very important factor in the formation of the Black soil.

Black soil has a thick black-colored A horizon very high in humus content. A great deal of active alumina produced as a weathering product of volcanic ash, parent material of the Black soil, is regarded as the humus bearer.

Classification of alpine soil

The central mountainous region of Japan is divided into a hilly zone

(less than *ca.* 500 m above sea level), a low mountain zone (*ca.* 500–1,500 m), a subalpine zone (*ca.* 1,500–2,500 m), and an alpine zone (*ca.* 2,500–3,000 m). In the alpine zone, the mean temperature in July and August is about 10°C, and there is snow cover from September or October to May or June.

Ohsumi and Kumada (1971) classified the alpine soils in the Japan North Alps (alpine zone of the northern part of the central mountainous region) into four soil groups: Alpine podzol, Alpine grassland soil, Alpine meadow soil, and Skeletal soil.

1. Alpine podzol

The Alpine podzol corresponds to the Dry podzol. The horizon sequence is usually $A_0(F)$, A_2, B_1 (iron-accumulated), and B_2 horizon. It is widely distributed on convex areas and intimately associated with a creeping pine shrub and a heather-like shrub (*Vaccinieto-Pinetum pumilae* and *Arcterio-Loiseleurietum;* Suzuki, 1965).

2. Alpine grassland soil

This soil corresponds morphologically to Brown forest soil. In most cases, podzolization is not observed. It is distributed on concave slopes and adjacent flat sites and is associated with the Gentianetosum subassociation of *Arcterio-Loiseleurietum*, tall alpine herb shrub stands or a part of the chianophile plant community (Ohsumi, 1969).

3. Alpine meadow soil

This soil is composed of a thick (25 cm) humic horizon, a bleached horizon and an iron-illuviated horizon, underlain by impermeable clayey parent materials. The humic horizon is divided into upper peaty and lower mucky subhorizons. The latter is often imbedded with several sheets of humus-deficient mineral layers, one of them being a thin (*ca.* 0.3 cm) layer of Akahoya volcanic ash spewn from the Kikai caldera, 6,000–6,500 y.B.P. (Machida and Arai, 1978). The soil is now covered with alpine wet meadow vegetation (*Faurieto-Carietum blepharicarpae;* Suzuki, 1965) (Ohsumi, 1969).

4. Skeletal soil

Very gravelly and immature, this soil is associated with a large part of the chianophile plant community and *Minuarita arctica-Potentilla Matsumurae* community.

Formation of Alpine meadow soil

Referring to the climatic changes during the post-glacial epoch proposed by Tsukada (1967), the formation process of Alpine meadow soil is supposed to be as follows. Accompanying the disappearance of glaciers of the Würm Ice Age, the soils in the alpine zone were scraped away and the C horizon was exposed. On the impermeable clayey C horizon deposited on

flats or gentle slopes, peat soils developed and readily transformed into muck soils under the warm and humid climate prevailing during the RII period (9,500–4,000 y.B.P.). Akahoya volcanic ash fell during the warmest time of the RII period. Thereafter, the climate became cooler during the RIIIa period (4,000–1,500 y.B.P.) and the transformation of peat to muck has since been prevented. The formation of the thick humic horizon was accompanied by gley podzolization. Materials of mineral layers in the mucky subhorizon were probably supplied from adjacent upper slopes by heavy rains during the RII period.

1.3. General Discussion on Soil Forming Process with Special Reference to Organic Matter

Kononova (1966) outlined briefly and systematically the importance of organic matter in soil formation and the natural factors of humus formation, such as plant cover, soil microorganisms, hydrothermal conditions, chemical and physico-chemical properties of soils. Here, as a complement to her description, the roles of organic matter and organisms in the soil-forming process and the transformation of organic matter are discussed.

Soil, organisms, and organic matter

Without living organisms and organic matter, the birth of soil is not possible. Soil is formed when living organisms settle in and work on inorganic parent materials (weathering products of rocks), and organic debris is incorporated into the inorganic materials. As the soil is the product of interaction between inorganic components and organic components, therefore, both weathering products and organic debris should be regarded as parent materials. If solumn refers to the A and B horizons and the C horizon is their parent material, then A_0 horizon must be a parent material too.

The first organisms invading the C horizon which is exposed by heavy rains, landslide, erosion or human change are blue-green algae, autotrophic bacteria, spores, seeds, and others. In the suburbs of cities in this country, leguminous plants such as arrowroot, bush clover and vetch flourish rapidly in abandoned residential areas. Grasslands develop with time, shrubs invade, and forests finally stand as a climax under the temperate humid climate prevailing in Japan. During these plant successions, the C horizon differentiates *via* A-C horizons to A-B-C horizons, and Brown forest soils are formed.

Generally speaking, the roles of organisms and organic matter in this soil formation process may be expressed as follows. Plants supply organic matter to soil in the forms of leaves, stems, twigs, seeds, trunks, roots, *etc.*.

Although a large part of the organic matter is decomposed by soil organisms on and in the soil, a small part of it is humified and remains there. The decomposition and humification of plant remains vary with plant species and their organs as well as soil conditions.

Various organisms take part in the decomposition of organic matter, soil animals such as earthworms, enchytraids, springtails, mites, and microorganisms such as fungi, actinomycetes, and bacteria. Their activities vary with soil conditions. Soil organisms and their metabolites are also decomposed or humified. Some kinds of organic matter synthesized by soil microorganisms and non-easily decomposable plant constituents may be incorporated into humus.

Although the ultimate decomposers are microorganisms, the role of soil animals is important and should not be ignored. For example, decomposition and humification will be delayed remarkably if the crushing and mixing of plant remains by soil animals do not occur beforehand.

The term decomposition is used here together with humification; actually the author does not know whether humification includes decomposition or not. It is certain that the two actions occur simultaneously, and that decomposition proceeds by vital action under biochemical law. The author believes humification to be an abiotic action, not controlled by biochemical law or vital phenomena; the reason will be explained later.

In general, the role of the consumers, herbivorous and carnivorous animals, in ecosystems on soil formation may be negligible, because it is estimated that the biomass of producers (green plants) amounts to about 2 thousand billion tons, while that of consumers at most is several billion tons.

Transition from L layer via *F layer to H layer*

The transitional changes in the organic matter constituting the A_0 horizon on the surface of forest soils are a typical example of the humification process referred to by Hayes and Swift (1978) as microbial and chemical transformations of organic debris.

In the FES system, the A_0 horizon, plant remains accumulated on the surface of soil are divided into L, F, and H layers after Hesselman (1926). The L layer is the litter layer composed of fallen leaves and other plant organs which still preserve their original shapes. The F layer is the fermentation layer composed of plant remains partially crushed and rotted, but with tissues which are still recognizable. The H layer is the dark brown or black amorphous humus layer.

As illustrated in Chapter 7, the humification process of the L layer to H layer is characterized thus; (i) plant remains collapse to smaller and

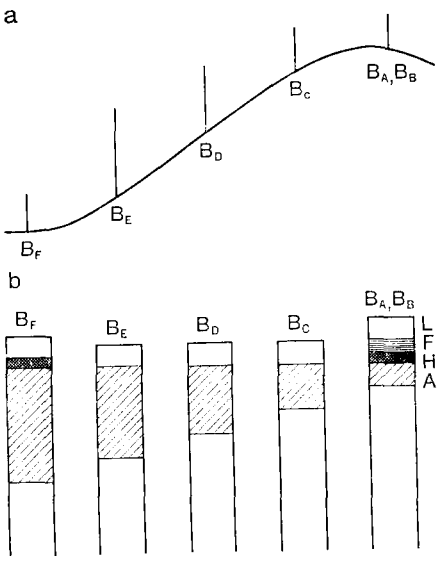


Fig. 1-1. Schematic representation of soil types (B_A—B_F) of Brown forest soil. a: topographic location; b: A_0 and A horizons. Vertical lines indicate growth of the tree.

finer fragments, (ii) their color darkens, (iii) their C/N ratios lower, and (iv) they are transformed to dark brown or black amorphous substances. Not only microorganisms but also soil animals participate in the humification process, so that the process should be regarded as physical, chemical, and biological transformation rather than "microbial and chemical."

The organic debris of the L and H layers may correspond approximately to Kononova's Groups I and II, respectively. The F layer may be regarded as the intermediate of both groups. In the author's opinion, however, it is practically impossible to separate the F layer into Groups I and II, making this grouping meaningless from the standpoint of humus chemistry.

As shown schematically in Fig. 1-1, the typical Brown forest soils are divided into 6 soil types, B_A–B_F. These soil types are thought to be formed depending on different moisture conditions which are mainly controlled by topography. Usually the B_A and B_B types are found near mountaintops and ridges, B_C, B_D, and B_E types are distributed along slopes in this order, and the B_F type is found on the lowest flats. As seen in the figure, the F and H layers develop well on the B_A and B_B types, but very poorly or not at all on the B_C, B_D, and B_E types. The B_F type is often accompanied by the H layer.

The rates of the transformation from the L layer *via* F layer to H layer

appear to differ from site to site, and to be controlled by the humidity of the air near the soil surface. The higher this humidity, the faster the transformation. The humidity of the air on the B_C, B_D, and B_E types is probably higher than that on the B_A and B_B types, which causes more rapid transformation of plant remains; consequently, the F and H layers do not develop. In the B_F type, extraordinarily high humidity may result in the formation of the H layer. Experiments are needed to verify these inferences.

The humification process in the A_0 horizon is universal, but not the true one, or perhaps better to say that it is the initial stage of humification, because this process in the A_0 horizon proceeds on the surface of the soil, and inorganic soil constituents take little part. The true humification process is the one which occurs in the interior of the soil. This is supported by the fact that HA produced during the humification process in the A_0 horizon is of the Rp(2) type according to the classification system proposed by Kumada *et al.* (1967) and modified by Kuwatsuka *et al.* (1978), while HA found in the interior of the soil belongs to other types, as described later.

Now, let us consider a little more precisely the humification process in soil.

Humification and weathering, and their interaction

As described above, the humification of organic debris is characterized by its fragmentation, the formation of humus, the lowering of C/N ratio, *etc.*. On the other hand, the weathering of rocks is characterized by physical disintegration, chemical decomposition, the formation of clay minerals, and the lowering of SiO_2/Al_2O_3 mol. ratio of residual materials. Thus, humification and weathering can be regarded as highly analogical processes with the following implications.

Organic substances which constitute living plants are synthesized and maintained by a vital force. They are unstable after the death of the plant. Minerals which constitute rocks have been synthesized under the high temperature and pressure of the lithosphere; they are also unstable on the surface of the earth. Humification and weathering are the stabilization reactions of both unstable substances under terrestrial conditions, *i.e.*, under normal temperature and pressure, and in the presence of water, oxygen, and carbon dioxide (Zolcinski, 1928, 1930).

As previously mentioned, soil formation begins when living organisms settle in the weathering products, the C horizon; weathering thus precedes humification. In the course of soil formation, however, both weathering and humification take place continuously, and biological activity and organic matter accelerate weathering (biological weathering). In some cases, the mode of weathering is fundamentally changed by the existence of plants.

One good example is observed in the weathering of volcanic ash. Volcanic ash is made up of fine mineral particles which are readily decomposed because of the large surface area. In the climate of Japan, mono- and divalent cations among weathering products are leached down, and allophane and imogolite are formed from the residual silica and alumina when plants do not grow there, as in a case of deeply deposited ash-fallout. With the existence of plants, however, silica is absorbed by them to form plant opal, and alumina and humus form an Al-humus complex which makes up the thick A horizon peculiar to the Black soil (Wada, 1977).

In general, weathering and humification processes proceed simultaneously in soil, interacting mutually, and resulting in the formation of humus-clay complexes. The quantity and quality of the complexes may vary with the organic and inorganic constituents and also with soil environments. Thus it is reasonable to consider that the real humification process is one which proceeds in the interior of the soil and is controlled by the physical, chemical, and biological properties of the soil.

Addition, translocation, and loss of organic matter

Plant remains are continuously added to soil, and are not only transformed but also translocated in the soil. A large part of the reaction products escapes from the soil. Whether or not the term humification involves all of these processes is moot, although in a broad sense it may. In this connection, Hayes and Swift (1978) stated that the synthesis and degradation of humus is a dynamic process which attains an equilibrium in a particular soil environment.

It would be better to say that some components of humus are labile and attain a dynamic equilibrium with a soil environment, and other components are stabilized by forming complexes with certain kinds of inorganic components. In any event, from the standpoint of pedogenesis a prime concern is to make clear the dynamics of SOM involving translocation as well as humification.

Soil environment as a soil-internal humification factor

The humification process in a broad sense is controlled by such soil-forming factors as climate, parent material, vegetation, relief, and time. It may be more practical, however, to replace these external factors with soil-internal factors. Here, soil-internal factors are tentatively defined as physical, chemical, and biological factors which constitute a soil environment for humification. At least these factors are involved: pH, exchangeable bases, amorphous Al, Fe, and Mn oxides, silicate clay minerals, moisture, aeration, drainage, and temperature; time is another important factor.

Total organic carbon and C/N ratio

These values have been widely used for characterizing soil. Apparently the determination is easy and seems to enable us to characterize various soils and compare them with each other. But the matter is not so simple when we consider the following: (i) The distribution of humus at a given soil sampling site is not always uniform horizontally and vertically. (ii) The division of the profile and the method of soil sampling may vary with each worker. (iii) The preparative method of a soil sample for analysis and the analytical method may vary with each laboratory. These factors can cause considerable variability in the data obtained.

In Table 1-2 are shown data of total carbon content and C/N ratio for the A horizon of Black (Bl) soil, Brown forest (B) soils (B_B and B_D types), and Red and Yellow (RY) soils.

In average values of total carbon content, the difference between the Bl soils and the RY soils is apparent, but both have wide ranges. The ranges of C/N ratio for the two soil groups are also wide, and it is difficult to distinguish between them. In total carbon and C/N ratio, the B_B soils and B_D soils can be distinguished from each other, but distinction cannot be made between the B_D soils and the Bl soils.

As illustrated here, the total carbon content and C/N ratio are not useful for distinguishing soils even in the order of soil group. It should be

TABLE 1-2
Total carbon content and C/N ratio of some soils in Japan.

Soil group	Number of samples	T-C%	C/N	Literature
Bl	10	13.3—4.80 8.72	24.5—13.2 15.5	Miki (1969)
Bl	46	26.3—2.73 11.8	27.5—12.4 20.0	Kobo and Oba (1974)
RY	9	4.39—1.01 2.25	23.6—6.7 15.0	Kanno (1961)
RY	9	2.99—0.95 1.86	25.6—8.8 17.7	Miki (1969)
B (B_B)	8	10.7—1.23 5.51	61.5—15.4 33.0	Ohta and Kumada (1978)
B (B_D)	24	18.9—4.75 10.1	18.1—13.0 15.3	Ohta and Kumada (1978)

Samples were taken from A horizon.
Figures of upper and lower tiers mean range and average values, respectively.

noted, however, that the soil samples used were fine soil (<2 mm) and contained varying amounts of crude plant remains. Analysis of very fine soil (<0.5 mm) or clay fraction (<2 nm) might have given different results. Further investigations are necessary to utilize total carbon and C/N ratio in soil characterization.

Comparative humus chemistry

In nature, humus, HA, FA, and humin are found in various environments: soils, sediments, waters, *etc.*. Let us take up HA for convenience.

"Unter Humus verstehen wir die Gesamtheit jener organischen Stoffe des Bodens, die under den jeweiligen Produktions- und Zersetzungsbedingungen des biologischen Standortes in characterische Weise zur Anhäufung gelangt" (Kubiena, 1948). Generalized, this states that HAs probably have their own individuality depending on their environment, which enables us to distinguish among them. At the same time, all have common features which enables us to call them HAs *en bloc*. In other words, the term HA which applies to all HAs found in various environments is a generic concept, and the same term used to refer to HA found in a given environment (*e.g.*, soil) is a specific concept. The same mode of thinking can be applied to the other terms mentioned. How do we define these terms as generic and specific concepts? It is natural that they should be defined in terms of chemistry.

To answer the question, HAs formed in different environments must be compared by various methods. Humus chemistry in this field may be called comparative humus chemistry. The publication "Humic Substances in Soil, Sediment and Water" (Aicken *et al.*, 1985) is a fine contribution to this subject. The development of comparative humus chemistry will contribute to the progress of organic geochemistry and each of its branches.

Chapter 2

Classification of Humic Acids

One of the main features of this humus chemistry is that HAs were divided into several types and their various properties studied on the basis of the classification system.

Before World War II, Springer (1938) and Simon and Speichermann (1938) proposed their own individual analytical methods of humus composition, both involving an HA classification system. Springer set up the following series of HAs: Humoligninsäuren (dem Lignin nahestehend, in Azetylbromid noch löslich)→Lignohuminsäuren (Huminsäuren mit Übergangscharakter, in Azetylbromid bereits unlöslich)→echte Huminsäuren des Kasselerbrauntypus→echte Huminsäuren des Schwarzerdetypus. The first three were generically called brown HAs, because it was supposed that they were closely related structurally and genetically, while the last named gray type was differentiated from them. Simon, meanwhile, divided HAs into echte Huminsäuren and Rotteprodukte (Humolignin or Humoligninsäuren), and subdivided the former into types A and B.

Although the terms gray and brown HAs are still seen here and there in the literature, the classification systems proposed by Springer and Simon seem to have been largely ignored by researchers. In the opinion of the author, however, classification is essential to the study of the complex and diverse HAs.

One of the first and most natural classification criteria should be their UV- and visible absorption spectra. Because HAs are brown substances, the brown color is one of their intrinsic attributes. Springer and Simon also adopted optical properties as the main criteria.

Soon after World War II when this humus study began, the available literature was mostly limited to the German scientists' papers. After a few

years of investigation, this author felt: (i) These earlier studies were of great value, because they had succeeded in showing clearly that the status of soil humus is diverse, and varies with the kind of soil. The diversity is exhibited not only by the differences in quantities of HAs and FAs, but also by the diverse optical properties of HAs and the different combinations of humus and soil inorganic components. (ii) Their optical properties should be useful not only as criteria for classifying HAs but also as a guiding principle for elucidating their nature and properties. (iii) A then widely used Pulfrich photometer (a type of filter photometer) was unsuitable for accurate determination of an absorption spectrum, and a spectrophotometer should be used. A series of investigations on the optical properties, elementary composition, and certain chemical and physico-chemical properties was then carried out from 1951 to 1959 using a Beckman Model DU spectrophotometer (Kumada, 1955a, b, 1956a, b, 1958; Kumada and Aizawa, 1958).

2.1. Studies on the Optical Properties of Humic Acids

In these investigations, HA samples were obtained from the A horizons of Black soils, Ap horizons of paddy soils (arable soils reclaimed for cultivation of rice plants), peat layers collected from peat soils at different depths, A_1 and B_2 horizons of a Podzol, *etc.*. The methods of Springer (1938) and Simon and Speichermann (1938) were followed.

Springer's method From soil samples either pretreated with 5% HCl at 70°C for 30 min or not, HAs were extracted by treating with 0.5% NaOH at boiling temperature for 30 min (Springer used for 1 hr). In these HAs, the one extracted after acid pretreatment was designated as SrL-HA, and other as L-HA.

Simon's method HAs were extracted by treating with N/8 NaF or NaOH at room temperature for 48 hr. They were designated as F-HA and the OH-HA, respectively, and as Si-HA collectively.

The HAs thus obtained were dissolved in 0.1% NaOH and ultrafiltered through a collodion membrane. The filtrates were acidified with HCl, transferred to a filter paper, washed successively with dilute HCl and water, then air-dried and pulverized. They were then separated into A and B types according to the method proposed by Simon and Speichermann (1938). That is, HAs were dissolved in an adequate amount of 1% Na acetate and an equal volume of 1 N $MgSO_4$ was added. The $MgSO_4$-precipitable and non-precipitable fractions were separated, and designated as A and B, respectively (*e.g.*, OH-A-HA, F-B-HA).

Determination of absorption spectra HAs were dissolved in 0.1% NaOH, and the absorption curves were promptly drawn in the region of

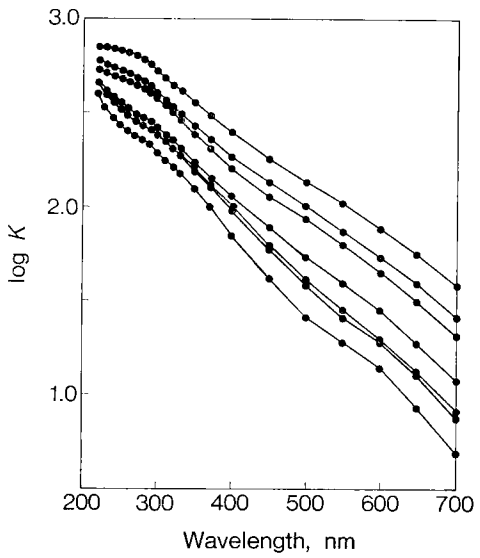


Fig. 2-1. Absorption spectra of L-humic acids.

220 to 700 nm. These curves were expressed as log K-λ curves of 1% solution of the HAs, where K and λ were optical density and wavelength, respectively.

The absorption curves of L-HAs (1% HA solution in 0.1% NaOH) are shown in Fig. 2-1. The optical density was large at the shortest wavelength and decreased toward longer ones. No distinct absorption band was observed in any wavelength region. The curves were similar in shape and rarely crossed each other. Quite similar situations were observed for the SrL- and Si-HAs.

The absorption curves in this figure indicate that the larger the optical density of an HA, the smaller is the inclination of the curve to the axis of wavelength. This was confirmed by the findings that there existed highly significant (0.1% level) negative correlations between the values of $K_6^{1\%}$ (optical density at 600 nm of 1% solution) and $\Delta\log K$ ($\log K_{400} - \log K_{600}$) not only for L-, SrL-, and Si-HAs, respectively, but for all the HAs, as shown in Fig. 2-2. Accordingly, it may be reasonable to adopt $K_6^{1\%}$ and $\Delta\log K$ values as parameters for the characterization of HAs. It is stressed that, despite the differences in the method of extraction, the HAs obtained can be uniformly characterized by these values.

For the respective HAs obtained by the different methods of extraction, the following is noted.

(1) The $K_6^{1\%}$ value of L-HA from a soil tended to be somewhat smaller than that of SrL-HA from the same soil, but the $\Delta\log K$ values of the two

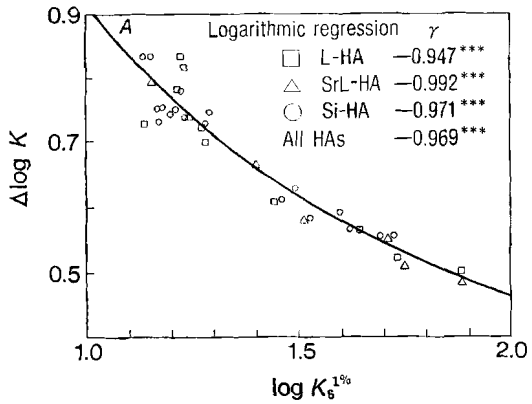


Fig. 2-2. Relationships between $\Delta\log K$ and $\log K_6 1\%$ values for L-, SiL-, Si- and all humic acids. *A*: logarithmic regression curve for all HAs.

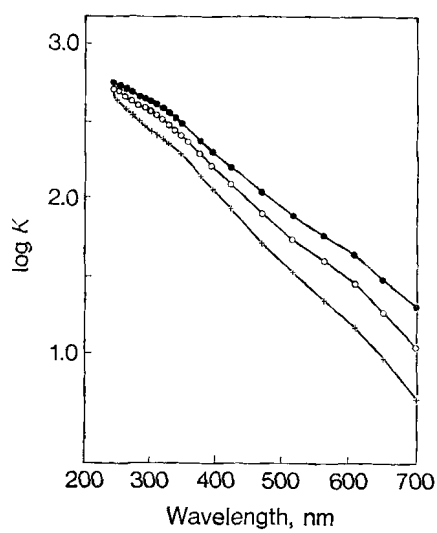


Fig. 2-3. Absorption spectra of F-A, OH-A, and OH-B humic acids. ●: OH-A (Takaho); ○: F-A (Takaho); +: OH-B (Takaho)

were nearly equal. Since the soils used in this investigation were all acid soils, decalcification by means of HCl treatment seemed to little affect the quality of the extracted HAs. If calcareous soils were used, such as Rendzinas or Grumusols, L-HAs would be considerably different from SrL-HAs.

(2) In the case of the Black soils and the paddy soils derived from Black soils, the amounts of OH-HAs were remarkably larger than those of F-HAs, and the OH-A-HAs showed larger $K_6^{1\%}$ and lower $\Delta\log K$ values, than those of the F-A-HAs, as illustrated in Fig. 2-3. This finding is remarkably different from that reported by Simon and Speichermann (1938).

Simon advocated that NaOH extracts part of the remains of rotten plants, while true HAs can be extracted with Ca-precipitable salts such as NaF and Na-oxalate. The author believes Simon's advocation to be appropriate to calcareous soils as shown later. In Black soils, however, the HAs are very similar to Simon's A type with respect to optical and other properties, and exist as Al-humus complex in soil, which is extractable with alkali.

Meanwhile, it is again stressed that the HAs obtained from different soils by different methods of extraction had $K_6^{1\%}$ and $\Delta\log K$ values of wide ranges, but highly significant negative correlations existed between them.

Degree of humification of HA The above finding on optical properties led to the following idea. Suppose the term humification connotes not only the formation of amorphous brown substances in soil but also the increase of brown color or blackish tone of the substances which the naked eye observes, *i.e.*, yellowish brown→brown→dark brown→almost black, the increase of brown color may then be said to express the progress of humification. This idea was born from the well-ordered shapes of the absorption curves of HAs and the regular relationship found between $K_6^{1\%}$ and $\Delta\log K$; these facts suggest that the chemical configurations responsible for light absorption are of the same quality, but differ quantitatively among HAs.

Consequently, a most important subject in humus chemistry is then to elucidate the chemical configurations responsible for brown color. This suggests the potential for answering the question of what humic substances are, and will be discussed later.

If the concept of progress of humification is permissible, the absorption curves in Fig. 2-1 may be said to exemplify it; their arrangement from the bottom upward exhibits the progress of humification. $K_6^{1\%}$ and $\Delta\log K$ values of individual HAs are considered to express the degree of humification. Furthermore, it is evident that various HAs can be arranged in the order of their degree of humification, *i.e.*, $K_6^{1\%}$ or $\Delta\log K$ values.

Although since World War II most researchers have ignored the absorption spectra of HAs because of their featureless character, the author considered this very featureless character the intrinsic attribute of HAs, and believed their surprisingly well-ordered absorption curves should indeed be the starting point of the study of HA. This idea is merely a working hypothesis, and its usefulness must be evaluated in light of the information which it has produced and will produce.

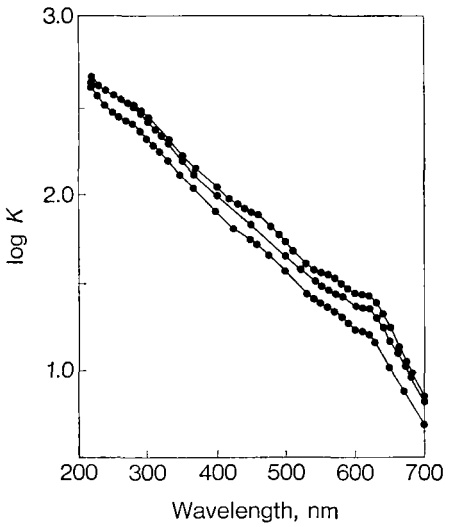


Fig. 2-4. Absorption spectra of P type L-humic acids.

In 1955 a special type of HA having absorption bands near 615, 570, and 450 nm (Fig. 2-4) was found and called P type, because it was first found in Podzols and was thought to be peculiar to this soil group. It is now certain, however, that the P type has worldwide distribution (see Chapter 4). In P type HAs, $\Delta\log K$ and $K_6^{1\%}$ values cannot serve as indices of the degree of humification because these values have been affected by the presence of the Pg fraction (see Sections 2.4 and 3.2.1).

2.2. Several Properties of Humic Acids

If HAs were arranged in the order of their degree of humification, changes in their various properties would also be recognized in accordance with the order. To verify this assumption, several properties of HAs, such as elementary composition, methoxyl content, cation exchange capacity, hydrolyzable nitrogen content, coagulation by adding electrolytes, and X-ray diffraction were examined (Kumada, 1955b, 1956a). Part of the results on the L-HAs are abstracted below; the results on other HAs were consistent with these.

As shown in Fig. 2-5, some regular changes in elementary composition were observed with the decrease of $\Delta\log K$ values, that is, with the progress of humification. The whole stage seemed to be roughly divided into early and later stages (lower and higher degrees of humification) with the turning

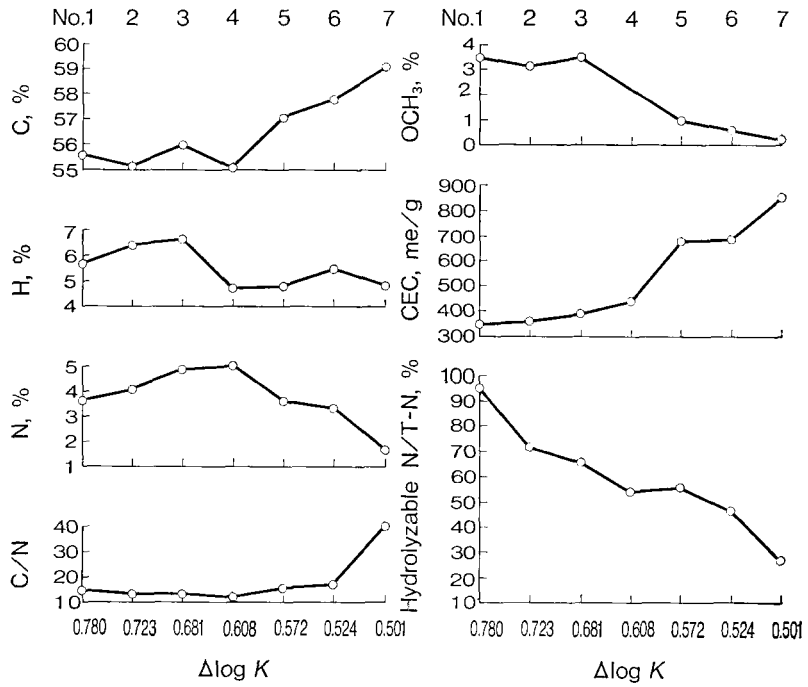


Fig. 2-5. Changes in several properties of L-humic acids with the progress of humification. C, H, N, and OCH_3: weight percent of moisture- and ash-free basis; CEC: me/100g HA, $Ba(CH_3COO)_2$ method; Hydrolyzable N: total-N in 6N HCl hydrolysate.

point sample No. 4. There were tendencies in the early stage for the carbon and methoxyl content and C/N ratio to remain unchanged and the nitrogen content to increase, and in the later stage, for the carbon content and C/N ratio to increase while the nitrogen and methoxyl content decreased. The hydrogen content of sample Nos. 1 to 3 was higher than that of Nos. 4 to 7.

CEC values increased with the progress of humification, suggesting the increase of acidic functional groups. On the other hand, the ratio of hydrolyzable nitrogen to total nitrogen was highest for the HA having the lowest degree of humification, and decreased remarkably with the progress of humification.

A coagulation experiment was conducted as follows: Five ml of 0.05 M CH_3COONa containing *ca.* 7 mg of HA was taken in each of several test tubes, and different amounts of 4 M NaCl or 2 M $MgSO_4$ were added, then a total 10 ml volume of each solution was made with water. The solutions were shaken for 1 min and allowed to stand. After 24 hr, turbidity of the

TABLE 2-1
Coagulation of L-humic acids by NaCl.

No.	Final concentration of NaCl (M)				
	0	0.2	0.4	0.8	1.6
1	K	K	K	K	N
2	K	K	K	K	N
3	K	K	K	K	N
4	K	K	K	K	N
5	K	K	N	F	F
6	K	K	N-F	F	F
7	K	K	F	F	F

K: clear; N: turbid; F: coagulated

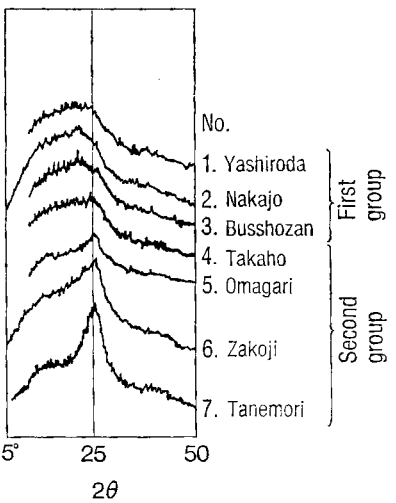


Fig. 2-6. X-ray diffraction diagram of L-humic acids.

solutions or coagulation of HAs was observed and recorded: K, transparent; N, turbid; and F, coagulated. The results illustrated in Table 2-1 show that the higher the degree of humification of the HA, the greater the sensitivity to the electrolyte; the HA was coagulated by the smaller amount of the electrolyte. This finding suggests that HA becomes more hydrophobic with the progress of humification.

As shown in Fig. 2-6, X-ray diffractograms of HAs were divided into two groups. In the first group, to which belonged HAs having lower degrees of humification, there existed a broad halo (so-called γ-band), suggesting their amorphous nature. In contrast, HAs belonging to the second group

TABLE 2-2
The (002) band of L-humic acids.

No.		2θ	d(Å)	Relative intensity
4	Takaho	24.7	3.60	43
5	Omagari	25.8	3.45	40
6	Zakoji	25.9	3.43	63
7	Tanemori	25.3	3.45	100

and having higher degrees of humification had a more or less distinct pattern at the position of $2\theta \fallingdotseq 26°$ with the above-mentioned halo, and the pattern became sharper, its shape symmetrical, and its intensity increased in proportion to the humification degree of the HA. Interplanar spacing in Å and intensity estimated from this pattern are shown in Table 2-2.

An obscure and feeble halo could also be observed at $2\theta \fallingdotseq 40$–$43°$ in sample Nos. 6 and 7 which have the highest degree of humification. Interplanar spacing roughly calculated from this angle was *ca.* 2.3 Å.

It is considered that interplanar spacings of 3.4–3.6 Å and *ca.* 2.3 Å of the HAs correspond respectively to the (002)- and (10)- bands of graphite. Consequently, HAs showing both these bands presumably have turbostratic structure. Here, turbostratic structure refers to stacked fractions of several layers with interplanar spacing of *ca.* 3.5 Å of condensed rings composed of several or more benzene rings. It is further presumed that HAs of the second group contain turbostratic structure which develops with the progress of humification. In other words, humification may represent an early stage of carbonization which occurs in soil. This X-ray diffraction analysis was rather preliminary and qualitative. A more quantitative analysis will be dealt with in Chapter 10.

The experimental results on the several properties of HAs clearly showed that these properties changed regularly in accordance with the degree of humification. Although the number of HA samples was limited, the findings may be enough to claim that optical properties can serve as a guiding principle in the study of HAs.

2.3. Classification of Humic Acids Based on Shape of the Absorption Spectrum and $\Delta \log K$

Although absorption curves of HAs were seemingly featureless, careful examination revealed that they could be divided into two groups according to their shapes as well as $\Delta \log K$ and $K_6^{1\%}$ values. In the HAs belonging to the first group, the curves exhibited bow-like shapes consisting of two broad convexes centering around 300 and 600 nm, and the $\Delta \log K$ values were

lower and $K_6^{1\%}$ values were larger than those of the second group. The HAs in the second group were nearly straight except for a shoulder-like absorption near 270 nm which was observed occasionally. In this group, the HAs originating from peats, rotten plant remains, farmyard manure, *etc.* were characterized by having even higher $\Delta\log K$ and smaller $K_6^{1\%}$ values.

Thus, HAs were classified as follows (Kumada, 1958b):

A type: HAs of the first group having $\Delta\log K$ value lower than *ca.* 0.6

B type: HAs of the second group having $\Delta\log K$ value of *ca.* 0.6 to 0.8

Rp type: HAs of the second group having $\Delta\log K$ value of *ca.* 0.8 to 1.1

P type: HAs having a peak or shoulder-like absorption near 615, 570 and 450 nm.

The terms A, B and Rp types originated from Simon's echte Huminsäuren Typ A und Typ B and Rotteprodukte, respectively. But the two classification systems are, in principle, quite different, because in Simon's classification, not only optical properties but also other properties were adopted as criteria, while in the author's, optical properties were the sole criterion.

2.4. Dr. M. M. Kononova's Criticism

It is inferred that HA of a soil sample is an assembly of fractions differing in optical properties, and the measured (*e.g.*, $\Delta\log K$ and $K_6^{1\%}$) values refer to the average values. Accordingly, even if the $\Delta\log K$ value of an HA corresponds to that of the B type, the HA may be separated into A and Rp types by dividing it into two fractions. This may occasion much discussion on the necessity and usefulness of classifying HAs.

In this context, Dr. M. M. Kononova wrote to the author on 25th September, 1972: "Your opinion concerning the nature and classification of humus substances is very interesting, though in some points I can't agree with you. Let me tell you my opinion.

"Humic acids of soils, peats, coals are extremely various and their diversity is not confined to "Grau" and "Braun" Humussäuren. But nonetheless they have several general common features (the presence of an aromatic ring, nitrogen as an obligatory component and functional groups including OH and COOH a. oth.); these features serve as a basis for their unification into a special group of humic acids. Based on several features (absorption spectra, aromatic ring), you introduce new terms for individual forms of humic acids, such as Rp, P, Pg. Undoubtedly, the number of new

terms will increase and this circumstance may lead to some misunderstandings.

"But this is still not the main thing. As you state in your letter, these new forms are usually extracted from incompletely humified plant residues or from soils enriched with them. In these cases, the 0.1 N NaOH or some other alkali solution causes inevitable oxidation and condensation of plant substances (such as some amino acids, phenols, tannins a. oth.) and their further co-extraction and co-precipitation with humic substances, but only as admixtures and not as their constituents.

"It is probable that the peculiarities you noted in absorption spectra were caused by these admixtures, but in this case, they cannot serve as a basis for their classification in specific forms of humic acids.

"Taking this into consideration, I think that now it is more correct to keep the general term "humic acids," noting their peculiarities (in elementary composition, color, IR spectra, functional groups a. oth.) for individual representatives.

"Now I want to say a little on the method of extraction of humic acids from incompletely humified plant residues. . . . Dr. I. V. Alexandrova showed that the treatment of the fresh clover leaves with 0.1 N NaOH resulted in an artificial formation of humus-like substances in these leaves. Therefore, I think that the extraction of humic and fulvic acids with 0.1 N NaOH, 100°C, 30 min—is a very drastic method! . . . ."

While appreciating her frank criticism, there seemed to be some differences in the mode of thinking and some misunderstanding, making agreement impossible.

Dr. Kononova appeared to recognize the diversity of HAs, yet avoided classifying them. This is one point of view, but there is another (already mentioned) viewpoint: classification is essential to aid understanding of HAs and their genesis.

Dr. Kononova's statement that the treatment of "fresh" (probably still green) clover leaves with 0.1 N NaOH resulted in an artificial formation of humus-like substances is understandable. However, Kumada and Suzuki (1969) studied the extracting conditions of rotted plant residues and found no evidence of the denaturation of HA optical properties under conditions of 0.1 N NaOH, 100°C, 30 min.

It is natural that HAs obtained from incompletely humified plant residues preserve more or less the features of the original plant components, some of which are reflected on the HA absorption spectra. Such HAs should be regarded as immature; they are included in the Rp(2) type in the present classification system proposed by the author (Section 2.6.). These immature

fractions must be carefully distinguished from mature, soil proper HAs (A, B, and Rp(1) types) to avoid confusion and misunderstanding on the nature of HAs.

The term Pg was given to the fraction of P type HA (Kumada and Sato, 1962) which contains dihydroxyperylenequinone nucleus responsible for the absorption bands near 615, 570, 450, and 280 nm (Sato and Kumada, 1967). In Dr. Kononova's opinion, the Pg fraction is an admixture and cannot serve as basis for setting up the P type. However, the P type has worldwide distribution; if this term cannot be adopted, we must refer to it as the HA having the absorption bands mentioned above or as that having Pg absorption. Thus, the terminology used is a matter of convenience.

The classification of HAs is admittedly very difficult compared with that of plants, animals, or low molecular organic compounds whose individual members can be identified. The difficulty is similar to that of soil classification. But HA classification must be attempted, and will be improved with the deepening of our understanding. We should not abandon classification.

2.5. IR Spectra of Humic Acids

The above classification is based on light absorption, so it is expected that the same grouping is possible according to the pattern of IR spectra. This expectation was confirmed experimentally (Kumada and Aizawa, 1958).

IR spectra of each type are illustrated in Fig. 2-7 and the characteristics follow:

A type: HAs belonging to this type have a fairly strong aromatic C-H stretching absorption band near 3.25 μm, and their two aliphatic C-H stretching bands near 3.4 μm are smaller than those of other types. The intensity of the absorption band near 3.8 μm assigned to O-H stretching of carboxyl group is strong. Two sharp absorption bands exist near 5.8–5.9 and 6.2 μm indicating the presence of C=O and C=C (including conjugated types) stretching vibrations, respectively. The latter may also be assigned to ring vibrations. The 6.1 and 6.6 μm bands obvious in the spectra of B and Rp types are absent or form a shoulder. There are two very broad bands in the region of 6.8–7.3 μm which may arise from aliphatic C-H deformation vibrations and phenols. A very broad and strong band is found in the region of 7.8–8.3 μm indicating the presence of oxygen-containing groups such as phenols, ethers, and quinones. Towards longer wavelength region, the absorption decreases fairly abruptly.

B type: The intensities of the two absorption bands near 3.4 μm due to aliphatic C-H stretching are very strong and sharp, while the 3.25 μm

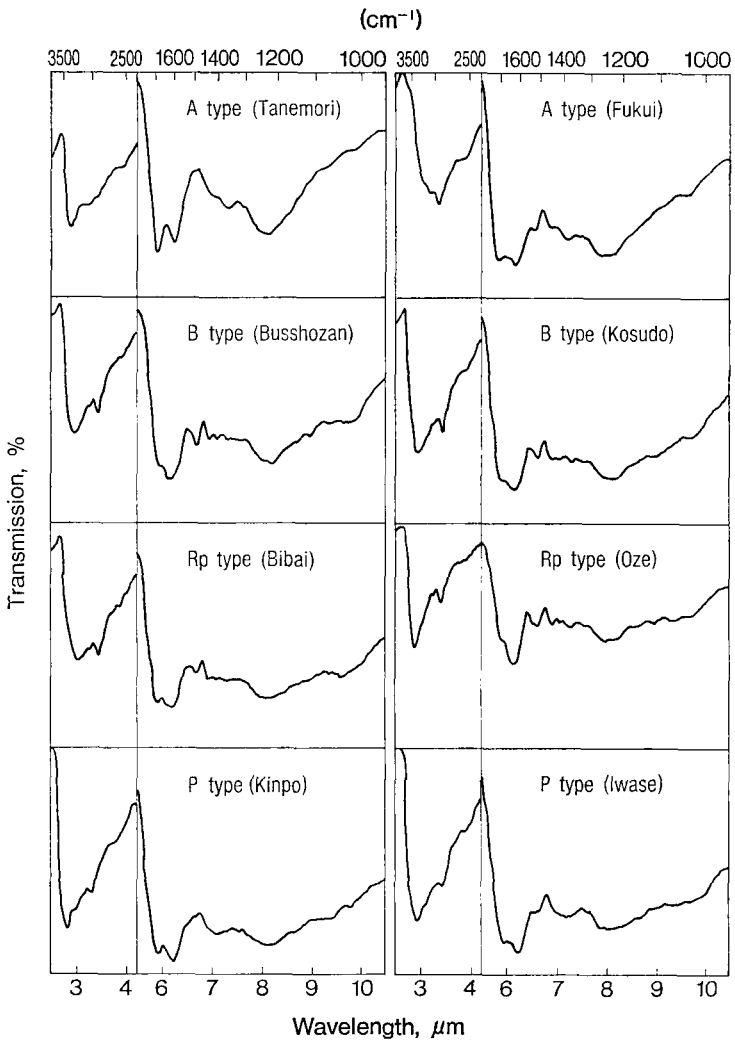


Fig. 2-7. Infrared spectra of humic acids.

band is absent and the 3.8 μm band is weak. The absorption band near 5.9 μm is weak and, in most cases, merely a shoulder. Besides the strong 6.2 μm band, another is found as a shoulder near 6.1 μm and a pronounced band is present near 6.6 μm. These latter two bands are considered to arise from the stretching vibration of C=C bands. Four fairly sharp bands are present near 6.9, 7.05, 7.2–7.3, and 7.5 μm, which probably arise from deformation vibration of aliphatic C-H groups. There is the strong, broad band at 7.8–8.3 μm. The band near 8.8 μm may indicate the presence of aliphatic

ethers. An absorption band occurs near 9.7 μm, presumably arising from C-O vibration of primary alcohols and/or C-O stretching of ethers.

Rp type: The pattern of the spectra of HAs belonging to this type is very similar in shape to that of B type, but some differences are noticed. The pattern of B and Rp types in the region of 2.8–7.5 μm is remarkably analogous to that of lignins (Suzuki and Kumada, 1972), which might support the hypothesis that HAs belonging to these types originate from lignin.

P type: The pattern of the spectra of P type HAs is similar in shape to that of A type; however, certain differences separate the two types: In P type, the intensities of 3.25, 3.4, and 3.8 μm bands are relatively weaker, the relative intensity of the 5.9 μm band to the 6.2 μm band is smaller, a shoulder-like absorption band is found near 6.5 μm, the pattern in the region of 6.9–7.3 μm is only a broad band, and the absorption in the region of 8.5–9.7 μm is fairly strong, as is true in B and Rp types.

As mentioned above, the 6.6 μm band was absent or weak in the A type, and distinct in the B and Rp types. In this connection, Kasatochkin and Zil'berbrand (1956) stated that in the IR spectra of HAs obtained from Podzols, an absorption band existed at 6.6 μm which was absent in those from Chernozems; this absorption band was related to the presence of various aromatic compounds having aliphatic side chains. They concluded that these HAs had to contain longer aliphatic chain structures than the HAs obtained from Chernozems.

2.6. Present Classification System of Humic Acids

In 1967, Kumada proposed a novel method of humus composition analysis (Section 6.1.). On that occasion a classification system of HAs was also proposed (Kumada *et al.*, 1967). This new system grouped HA into five main types, *i.e.*, A, B, Rp, P_0, and P, according to their position on the two coordinates having *RF* and $\Delta\log K$ as axes and divided as shown in Fig. 2-8. Here, *RF* is defined as $K_{600} \times 1{,}000/c$, where K_{600} and c mean optical density at 600 nm of an HA solution and ml of 0.1 N $KMnO_4$ consumed by 30 ml of the HA solution, respectively.

Absorption spectra of A, B, and Rp types are schematically shown in Fig. 2-9. The Rp type is subdivided into Rp(1) type originating from A and B horizons and Rp(2) type from A_0 horizon, rotted plant remains, farmyard manure, compost, *etc.* (Kuwatsuka *et al.*, 1978).

Absorption curves of P_0 and P types are shown in Fig. 2-10. The P type is subdivided roughly into $P_{\pm}$, P_{+}, P_{++}, and P_{+++} types, depending on the intensities of absorption near 615, 570, and 450 nm. The P_0 type refers to the HA which is located in the same area as the P type but has no absorp-

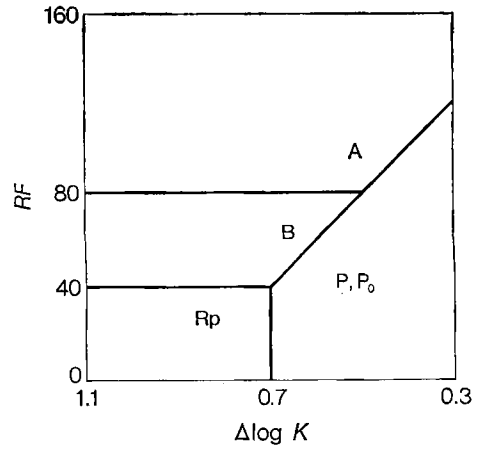


Fig. 2-8. Classification diagram of humic acid.

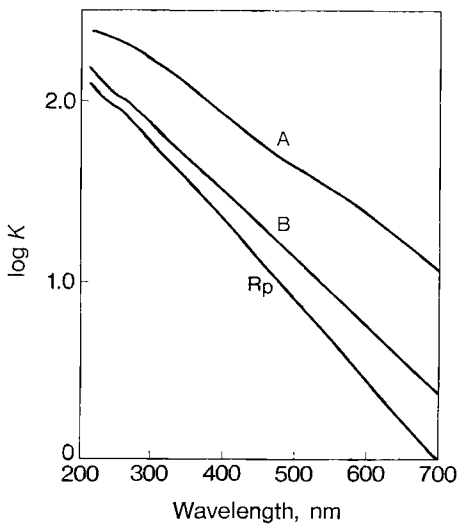


Fig. 2-9. Absorption spectra of humic acids (A, B, and Rp types). Concentrations are arbitrarily chosen.

tion band. The HAs which are located outside the P type area and have the same absorption bands as the P type are subdivided as in the case of P type, *e.g.* $Rp_{\pm}$, A_{+}.

Among Rp and P_0 types, there are some whose absorption spectra suggest the presence of lignin- or tannin-like structure as illustrated in Fig.

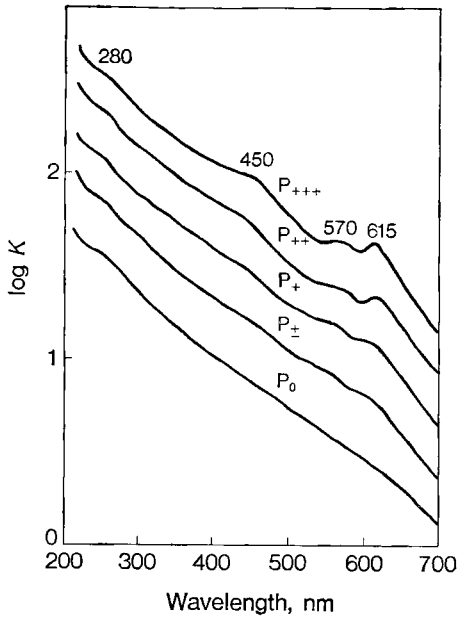


Fig. 2-10. Absorption spectra of P_0 and P type humic acids.

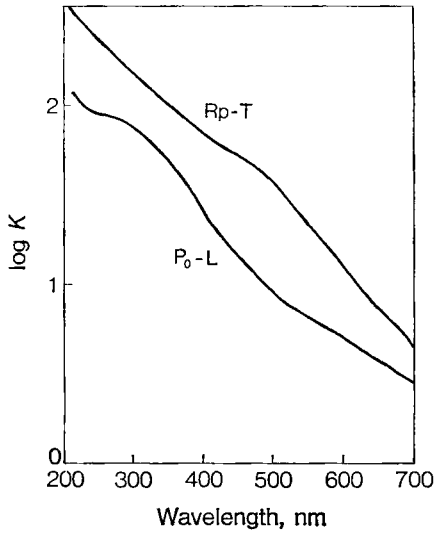


Fig. 2-11. Absorption spectra of Rp-T and P_0-L type humic acids.

TABLE 2-3
Humic acid classification systems.

Simon and Speichermann (1938)	Springer (1938)		Kumada (1981)
Humolignin (Rotteprodukte)	Humoligninsäuren		Rp type { Rp (2) type / Rp (1) type
	Lignohuminsäuren	Braunhumin-säuren	
echte Huminsäuren Typ B	echte Huminsäuren (Kasselerbrauntypus)		B type
echte Huminsäuren Typ A	echte Huminsäuren (Schwarzerdetypus)	Grauhumin-säuren	A type
			P_0 type
			P type $P_{\pm}$~+++ types

2-11. Absorption spectra of the former and the latter are respectively characterized by a convex absorption ranging from 280 to 400 nm and a shoulder-like absorption near 500 nm often accompanied by other shoulders. They are differentiated by subscripts L and T, respectively, *e.g.*, Rp-L, P_0-T. Several properties of Rp(2) type HA were reported by Suzuki and Kumada (1972).

Comment

In some Rp type HAs, *e.g.*, those originating from incompletely rotted leaves and straw, absorption bands at 660 nm and near 400 nm are often seen due to the existence of chlorophylls or pheopigments. But the bands seem to disappear rather rapidly with the progress of rotting in terrestrial conditions.

In HAs obtained from lake and marine sediments, absorption curves are often characterized by an absorption band near 400–410 nm, corresponding to the Soret band of porphyrins, and suggest their phytoplankton origin (Watanabe, 1969; Ishiwatari, 1973). It is therefore supposed that chlorophyll-derived pigments are unstable and readily decomposed under terrestrial conditions, but are fairly stable in lake and marine sediments.

A comparison of the classification systems of HAs

The classification systems of HAs proposed by Simon, Springer, and the author are tabulated in Table 2-3. Though similar, the criteria for classification are considerably different.

Chapter 3

Spectroscopic Characteristics of Humic Acids and Fulvic Acids

In the preceding chapter, the HA classification system proposed by the author was described and a brief historical review of his studies on HAs emphasized the following.

Absorption spectra of HAs in the UV- and visible region seem to have been largely ignored because of their featurelessness. But this very featurelessness is the character of the brown color, one of their intrinsic attributes, and serves not only as the basis of their classification, but also as the guiding principle of studies about them. There also exists a group of HAs called P type, which have specific absorption bands near 615, 570, and 450 nm.

It may be appropriate in this chapter to discuss more precisely the spectroscopic characteristics of HAs and FAs, the determination and characterization of their UV- and visible absorption spectra, and their ionization difference and reduction difference spectra. The preparation methods of HA and FA samples are described and some related problems commented on. Finally, a working hypothesis on the mechanism of light absorption of HAs is introduced.

3.1. Preparation of Samples

Preparation of HA and FA samples involves many complicated and troublesome problems, some of which will be mentioned later. First, the method used in the author's laboratory is described. To obtain HA samples the procedures of Springer and Simon were adopted, as mentioned previously. Then, a new method was explored, which in principle corresponded to the extraction and separation stages of HA in the humus composition analysis proposed at that time, but was upgraded to get samples at least of the order

of grams. Since then, detailed procedures of the method have been gradually modified, and the present method (Kumada, 1985a) is here described, in which preparation of a colored fraction of FA is involved. For convenience sake, this is called the Nagoya method.

Procedure

Humus is extracted from a mixture of soil (air-dried, <0.5 mm) and extractant (organic carbon to extractant ratio, 1:300, w/v) at room temperature for 24 hr. As extractant, 0.1N NaOH or a mixture of 0.1N NaOH and 0.1M $Na_4P_2O_7$ is used. In some cases, successive extraction with 0.1N NaOH and 0.1M $Na_4P_2O_7$ is useful. The humus extract is separated from the residue by centrifuging at $10,000 \times g$ for 15 min after adding an amount of NaCl equivalent to 3% of the extractant. HA is recovered by adjusting the pH of the extract to 1.0 with HCl(1:3). After repeated dissolution with 0.1N NaOH or 0.1M $Na_4P_2O_7$ and precipitation with HCl till the filtrate becomes almost colorless, HA is dialyzed against water, then freeze-dried. In the case of successive extraction, the first humus fraction is extracted with 0.1N NaOH, then the second one with 0.1M $Na_4P_2O_7$ from the residue by repeating the same procedure.

To the combined acid filtrates, polyvinylpyrolidone (PVP) is added (PVP 1 g to 100 ml) according to the method proposed by Lowe (1980). After intermittent stirring for 1 hr, the mixture is transferred to a filter paper and washed with HCl (pH 1.0). The colored substances adsorbed on the PVP are eluted with 0.1N NaOH. After acidification of the alkaline eluant with HCl, the eluant (FA) is dialyzed against water, then freeze-dried. Thus, FA here refers to the PVP adsorbed fraction, that is, the colored substances in what has been designated in previous reports as FA. In some cases, the alkaline eluants may produce considerable amounts of precipitate when acidified to pH 1.0. The acid-precipitable FA_1 and non-precipitable FA_2 fractions are dealt with separately.

Comments

(1) Preparation of soil sample

Usually air-dried soils are used for extraction of humus. Denaturation of organic matter by air-drying of wet soils may be mostly negligible, but this is not necessarily true in the case of peat soils. Air-dried and sieved soils contain more or less fine roots and other crude organic debris. The <2 mm soils may contain larger amounts of crude organic debris than the <0.5 mm soils. The crude organic debris may be a source of soluble humus, but the author does not know the extent of the part it may play in this. A method for removing beforehand a large part of crude plant debris from soil may be needed.

(2) Extraction

As extractant, 0.1 N NaOH, 0.1 N NaOH + 0.1 M $Na_4P_2O_7$ or 0.1 N NaOH followed by 0.1 M $Na_4P_2O_7$ (successive extraction) have been used. In the case of acid and base-deficient soils prevailing in this country, 0.1 N NaOH can extract a large part of soluble humus, but a mixture of 0.1 N NaOH and 0.1 M $Na_4P_2O_7$ (1:1, v/v) have been used for soils with a wide range of pH values. A mixture of 0.1 M NaOH•0.1 M $Na_4P_2O_7$ (0.1 M with respect to each component) recommended by Kononova (1966) has been used widely.

(3) Soil:extractant ratio

The amount of extractable humus varies with soil:extractant ratio. Kobo and Oba (1957) reported that the maximum yield was obtained at the organic carbon: extractant (0.5% NaOH) ratios of 1: 300–400. Kawada (1972) compared the ratio of 300, 500, and 700, and recommended the ratio of 1:500. Recently, Ito (1985) obtained maximum yield at the ratio of 1:300. Anyhow, it is almost meaningless to fix a soil:extractant ratio, because the organic carbon content varies with the soil.

(4) Temperature and time

Referring to the experimental results reported by Springer (1938) and Hock (1936/1937), the author formerly adopted 100°C and 30 min for extraction of humus. But heating a large amount of soil was not practical, so a room temperature or 30°C and 24 hr were substituted. In quantitative experiments using a small amount of soil sample, the yield of extractable humus was in the order of 100°C, 30 min > room temperature, 48 hr > room temperature, 24 hr (Kumada and Ohta, 1963).

Studies reported by Bremner (1950) and Swift and Posner (1972) are often cited as illustrations of the degradation or denaturation of humus by treating it with alkali. Certainly treatment with alkali causes some degradation of humus, but the degree may depend on the conditions of treatment quantitatively and qualitatively. For instance, Swift and Posner (1972) treated HA for 30 days with normal NaOH under O_2. This treatment condition seems to be rather drastic compared to that usually used for the extraction and purification of HA.

The effect of the exclusion of oxygen by bubbling nitrogen gas has not been tested. However, the author's procedures called for a mixture of soil and extractant to fill a narrow-necked bottle to the neck, and this was stoppered tightly during the extraction period.

(5) Separation of humus extract

Humus extract was usually separated from soil residues by centrifuging. Data reported by Schnitzer *et al.* (1981) illustrated that the quantity and quality of the extract varied remarkably with the conditions of centrifugation, *i.e.*, rpm (*g*) and time. A mixture of soil and extractant contains not only

true and colloidal humus but also colloidal and suspensible humins or humus-clay complexes and inorganic particles of various sizes. The stronger the centrifugal force and the longer the operating time, the finer and lighter are substances remaining in the extracts. The boundary between extractable humus and non-extractable humus and other residues is obscure, and may largely depend on prevailing conditions.

To coagulate humins and humus-clay complexes, NaCl or Na_2SO_4 is added and the extract is obtained by centrifuging ($10{,}000 \times g$, 10–20 min). The experimental basis for justifying these conditions is, however, rather poor.

(6) Purification of HA

Here, purification means the removal of ash and FA and is achieved by the repetition of dissolution by NaOH or $Na_4P_2O_7$ and precipitation by HCl; HF-HCl treatment may sometimes be necessary to get the ash content lower than 1%. It seems almost impossible, however, to remove FA completely from a large amount of HA. Also, there may be the possibility that FA is newly formed by the degradation of HA.

(7) Purification of FA

The procedure to obtain the PVP-adsorbed fraction is very easy. The author's experience showed the ash content of such fractions to have a wide range, some of them being very high. As discussed later, it is extremely difficult to devise a purification method in which the loss of FA is kept at a minimum. Another problem was the observation of irreversible adsorption of FA on the PVP. As Lowe (1975) stated, the PVP-non-adsorbed fraction can be recovered by ultrafiltration if low molecular fractions are ignored.

(8) Drying of HA and FA

Freeze-drying is now readily available. In general, freeze-dried HAs exhibit two forms: one is black and bright, hard angular granules, and the other is yellowish brown or brown, very thin filaments or films. The amounts of the latter are, in most cases, negligible, but are sometimes large. Separation of the two fractions is easy. When the mixture mounted on a filter paper is washed with water, the former remains on the paper, and the latter is immediately passed through to form a brown filtrate. The filtrate produced precipitates at pH 1.0; thus the precipitable fraction may be called water-soluble HA. The $\Delta \log K$ value of its alkaline solution is slightly higher than that of the above mentioned granules, but distinctly lower than that of FA. Accordingly, these two fractions seem to be different from each other, although no further examination has yet been carried out.

3.2. Spectrometric Characterization of Humic Acids and Fulvic Acids

Since the author first used a spectrophotometer in 1951 to determine the absorption spectra of HAs, remarkable progress has been achieved on this kind of instrument, and there are now instruments which enable the automatic identification and recording of absorption curves and optical density values at definite wavelengths. The author's recent investigation on the spectrophotometric characteristics of HAs and FAs (Kumada, 1985a) is outlined below.

3.2.1. Ordinary absorption spectra

HA and FA samples were obtained from four Canadian and two Japanese soils. General soil properties of the Canadian soils (#1–4) are listed in Table 3-1 (Lowe and Kumada, 1984); these were used to verify the appropriateness of the information on the HAs of soils in Japan to those of foreign soils. Soil #5 (Inogashira) was the A_1 horizon of a Black soil. Soil #6 (Tsubame) was a buried muck-like humic horizon under a Brown forest soil located on a ridge *ca.* 1,200 m above sea level. Although the genesis of the horizon is not well known, it is presumably the subalpine type of muck-like subhorizon of Alpine meadow soil. The soil was characterized by its content of many black hollow spherules (diameter, <7 mm), which were sclerotia of *Cenococcum graniforme*?, a main source of P type HA.

HA and FA samples were prepared according to the procedures described in Section 3.1.

Referring to the results of humus compostion analysis (Section 6.2.), the extractants were selected as follows: In soil #1, humus was extracted successively with 0.1 N NaOH and 0.1 M $Na_4P_2O_7$, and the two fractions

TABLE 3-1
General properties of Canadian soils used[a].

Sample No.		Horizon	HM[b] (%)	pH[c] ($CaCl_2$)	Total C[d] (%)	Total N[e] (%)	C/N
1	Orthic Dark Brown (Chernozemic)	Ah	1.87	6.76	1.95	0.206	9.5
2	Black Solonetz	Ah	4.23	5.59	7.12	0.795	9.0
3	Orthic Ferro-Humic Podzol	Bhf	4.93	4.30	5.61	0.254	22.1
4	Regosolic Static Cryosol	Ah	6.19	6.53	11.3	0.663	17.0

[a] Air-dried soil basis. [b] Hygroscopic moisture, dried at 105°C, 24hr. [c] 1:2 in 0.01 M $CaCl_2$.
[d] Induction furnace. [e] Semi-microkjeldahl. [f] Fisher analyzer.

were designated #1 OH and #1 P. For soil #2, a mixture of 0.1 N NaOH and 0.1 M $Na_4P_2O_7$ was used as extractant, while for soils #3 to #6, 0.1 N NaOH was used.

In soils #3, 4, and 6, the alkaline eluants of PVP-adsorbed fractions produced considerable amounts of precipitate when acidified to pH 1.0. The acid-precipitable FA_1 and non-precipitable FA_2 fractions were dealt with separately.

Preparation of sample solutions

HA and FA sample solutions can be readily prepared by mixing sample and solvent (0.1 N NaOH). In this investigation, however, the following procedure was tentatively adopted to secure rapid and complete dissolution of the sample. A mixture of suitable amounts (5–40 mg) of the sample and a small volume of 0.1 N NaOH was passed through a filter paper (Toyo Roshi No. 6) and the paper was washed with 0.1 N NaOH till the volume of the eluate reached 100 ml.

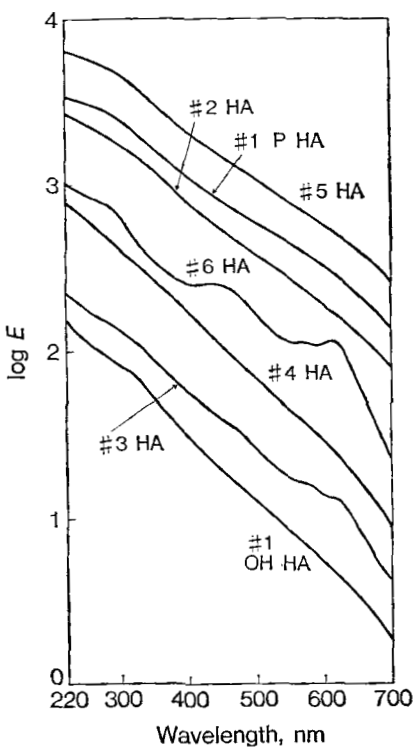


Fig. 3-1. Absorption spectra of humic acids.

Absorption curves in the region of 220 to 700 nm were drawn using a Shimadzu UV-240 spectrophotometer equipped with OP1-2, and optical densities at 10 nm intervals were automatically recorded. Determination was conducted immediately after the preparation of sample solutions. The sample solution as well as dilute sample solutions (the original solution was diluted 2.5 to 5 times) was used to obtain reliable values over the entire wavelength region. Beer's law held within the range of *ca.* 0.08 to 2.0 of the optical density.

Absorption spectra were presented in terms of log E *vs.* wavelength curves. To identify parameters suitable for characterizing the absorption spectra, $E_6^{1\%}$, $E_4^{1\%}$, $E_{2.5}^{1\%}$, and E_4/E_6 values were calculated, where $E^{1\%}$ is the optical density of the solution containing 1% HA- or FA carbon, and the affixed numbers 6, 4, and 2.5 correspond to 600, 400, and 250 nm, respectively.

The amount of the Pg fraction, 4,9-dihydroxyperylene-3,10-quinone (DHPQ) moiety of P type HA, was estimated by Sato's method (1974). The G_1 fraction separated from soil #6 (Kumada and Sato, 1980) was used as the standard Pg, whose $E^{1\%}$ values at 640, 615, and 590 nm were 115, 255 and 148, respectively.

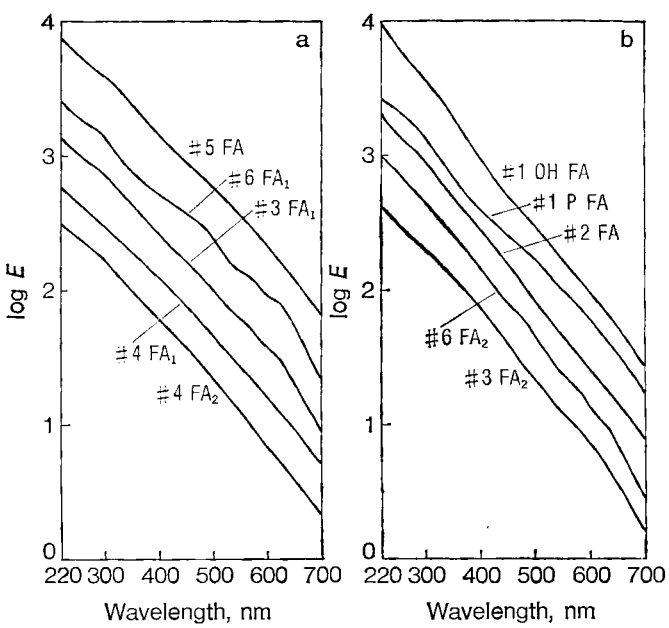


Fig. 3-2. Absorption spectra of fulvic acids. a: group 1; b: group 2.

Absorption spectra in 0.1 N NaOH

The absorption spectra of all samples in 0.1 N NaOH are shown in Figs. 3-1 and 2. Sample #6 HA showed 4 distinct absorption bands at 615, 570, 450, and 280 nm due to the dissociation of the DHPQ moiety. These bands will be referred to as Pg absorption hereafter. Pg absorption was observed in samples #6 FA_1 and FA_2 and also in #3 HA and FA_1, although the intensities varied appreciably. The shapes of the absorption curves of the other samples were almost featureless and nearly straight, and those of samples #5, #1 P and #2 HAs and #1 P FA corresponded to that of A type HA.

Even in cases when these almost featureless and nearly straight absorption curves showed Pg absorption, their intensities were not strong except for samples #6 HA and FA_1 and #3 HA. It was observed that the stronger the light absorption, the more gradual was the inclination of the curve. The $E_6{}^{1\%}$, $E_4{}^{1\%}$, $E_{2.5}{}^{1\%}$, and E_4/E_6 values and Pg contents as well as elementary composition and ash content of HAs and FAs are given in Table 3-2.

TABLE 3-2
Ash contents, elementary composition, and parameters of absorption spectra of humic acids and fulvic acids.

Sample		Ash (%)	C (%)	H (%)	N (%)	C/H	C/N	$E_6{}^{1\%}$	$E_4{}^{1\%}$	$E_{2.5}{}^{1\%}$	E_4/E_6	Pg (%)
#5	HA	0.75	58.50	3.96	3.77	14.8	14.8	86.2	297	863	3.4	0
#1 P	HA	2.50	57.79	3.58	3.68	16.1	15.7	88.9	305	902	3.4	0
#2	HA	1.60	57.45	3.72	5.17	14.7	11.1	62.8	266	780	4.2	0
#6	HA	5.22	57.06	4.73	3.13	12.1	18.2	76.2	184	622	2.4	7.8
#4	HA	1.64	56.13	4.70	3.70	11.8	15.2	24.8	144	486	5.8	0
#3	HA	10.4	56.02	4.74	3.45	11.8	16.2	46.2	186	648	4.0	1.8
#1 OH	HA	2.15	54.98	4.99	5.28	11.0	10.4	23.8	133	526	5.6	0
Group 1												
#5	FA	3.17	60.50	5.79	7.28	10.5	8.3	13.4	92.9	380	6.9	0
#6	FA_1	3.45	59.03	5.37	6.17	11.0	9.6	19.5	103	417	5.3	1.0
#3	FA_1	2.36	58.05	4.91	4.56	11.8	12.7	17.1	119	475	7.0	0.08
#4	FA_1	3.70	57.08	4.80	4.20	11.9	13.6	17.5	125	450	7.2	0
#4	FA_2	7.90	54.01	3.80	1.56	14.2	34.6	13.5	143	572	10.6	0
Group 2												
#1 OH	FA	16.7	55.64	5.89	7.75	9.4	7.2	3.8	38.6	246	10.2	0
#1 P	FA	38.1	52.96	5.03	5.60	10.5	9.5	13.4	85.0	436	6.3	0
#2	FA	13.1	52.52	4.34	3.69	12.1	14.2	9.7	103	516	10.6	0
#6	FA_2	5.88	52.38	3.48	1.52	15.1	34.5	10.5	111	622	10.6	0.08
#3	FA_2	1.46	51.41	3.31	0.70	15.5	73.4	12.1	131	621	10.8	0

Ash, C, H and N, weight %, organic matter basis. For $E_6{}^{1\%}$, $E_4{}^{1\%}$, $E_{2.5}{}^{1\%}$, E_4/E_6, and Pg, see text.

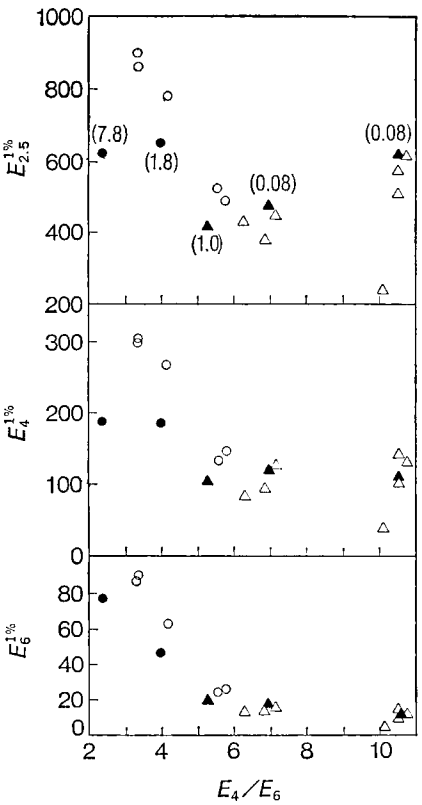


Fig. 3-3. $E^{1\%}$ *vs.* E_4/E_6 diagrams. ○ ●: HA; △ ▲: FA; ( ): Pg%. ● and ▲ indicate the samples which showed Pg absorption.

As shown in Fig. 3-3, individual components of HAs and FAs were distinguished from one another based on the $E_6^{1\%}$, $E_4^{1\%}$ or $E_{2.5}^{1\%}$ *vs.* E_4/E_6 diagrams, and the most conspicuous differences were observed on the $E_6^{1\%}$ *vs.* E_4/E_6 diagram. There were negative correlations between the $E_6^{1\%}$, $E_4^{1\%}$ or $E_{2.5}^{1\%}$ value and E_4/E_6 value for all HAs and the ones except for samples #6 and #3 HAs; the correlation coefficients for the former and the latter were, -0.88, -0.57 and -0.61, and -0.996, -0.987 and -0.995, respectively. It is evident that the larger the $E_6^{1\%}$, $E_4^{1\%}$ or $E_{2.5}^{1\%}$ value, the lower is the E_4/E_6 ratio, and a Pg content higher than 1.8% significantly affects the E values and/or E_4/E_6 ratios. The Pg content of 1% for #6 FA_1 may also affect the $E^{1\%}$ and/or E_4/E_6 values. No correlations between the $E^{1\%}$ values and E_4/E_6 ratios for FAs seem to exist, but this will be discussed later. It is noted that the relations between the $E^{1\%}$ values and the E_4/E_6 values for the HAs co-

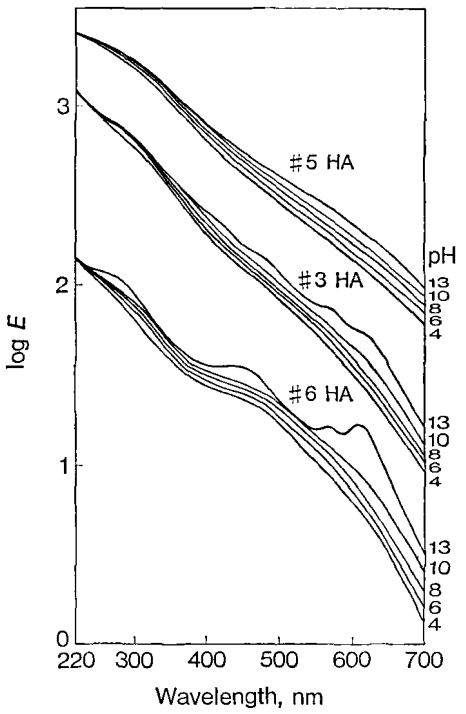


Fig. 3-4. pH dependency of absorption spectra.

incide in principle with those between the $K_6{}^{1\%}$ values and the $\Delta\log K$ values described previously.

pH dependency of absorption spectra

It is well known that the absorption of HAs and FAs is pH dependent. To validate this assumption quantitatively, sample solutions having pHs of 10, 8, 6, and 4 were prepared by adding dilute phosphoric acid (1:9) to the 0.1 N NaOH solution (pH$\simeq$13) used in the above experiments, and their absorption spectra were determined.

As illustrated in Fig. 3-4, the optical density at each wavelength tended to decrease with the value of the pH, and the longer the wavelength, the greater the decrease. This finding suggests that the lower the pH of the solution, the smaller becomes the $E^{1\%}$ value and the higher the E_4/E_6 ratio. In sample #3 containing 1.8% Pg, Pg absorption almost disappeared at pH 10, and the curve became nearly straight. In sample #6 HA containing 7.8% Pg, however, Pg absorption disappeared at pH 10, but two shoulder-like bands remained even at pH 4.

Comments

(1) In the absorption curves of HAs in 0.1 N NaOH, an absorption peak in the region of 220–230 nm is sometimes observed; this peak is not real but a deceptive one due to the change in the optical density of the alkaline solvent in the control cell by absorption of CO_2. In such a case, the solvent should be renewed.

(2) Absorption spectra are presented in terms of E *vs.* wavelength, log E *vs.* wavelength, or log E *vs.* log wavelength curves. The log E *vs.* wavelength curves are recommended because the absorption bands near 615, 570 and 450 nm are visualized most distinctly by this form and the shape of the curves is independent of the concentration of samples.

(3) The E_4/E_6 ratio proposed by Kononova (1966) has been used as a parameter for characterizing HAs and FAs. It is defined as the ratio of E at 465 nm to E at 665 nm determined in 0.02 or 0.05 N $NaHCO_3$ solution (pH 8.2–8.8). But the E_4/E_6 defined in the present investigation is more suitable for the following reasons.

(i) The wavelengths of 465 and 665 nm were selected from the filters equipped in a filter photometer, but the optical density at 665 nm is likely to give an inaccurate value, because the value actually measured is often too small.

(ii) As seen in Figs. 3-1 and -2, the linearity between 400 and 600 nm is better than that between 465 and 665 nm.

(iii) As described above, Pg absorption can be observed only when a solution with a high pH is used.

(iv) As Protonikova and Ponomareva (1967) recommended, the use of 0.1 N NaOH is convenient and gives the highest and most stable optical density value. It should be emphasized that determination of the absorption spectra is essential prior to the calculation of the E_4/E_6 value, because Pg absorption may affect the value significantly.

(4) Various pH solutions have been used to determine the absorption spectra of HAs and FAs, and the selection of pH is still debatable. The use of 0.1 N NaOH, however, is considered preferable, as mentioned above.

3.2.2. Relationships between elementary composition and spectral characteristics

As a means to examine the relationships between elementary composition and spectral characteristics, the diagrams shown in Fig. 3-5 were prepared. For HAs, high positive correlations were found between the $E_6^{1\%}$, $E_4^{1\%}$ or $E_{2.5}^{1\%}$ value and carbon content (γ: 0.91, 0.91, and 0.86), indicating that the light absorbability of HA increases with the increase of carbon content.

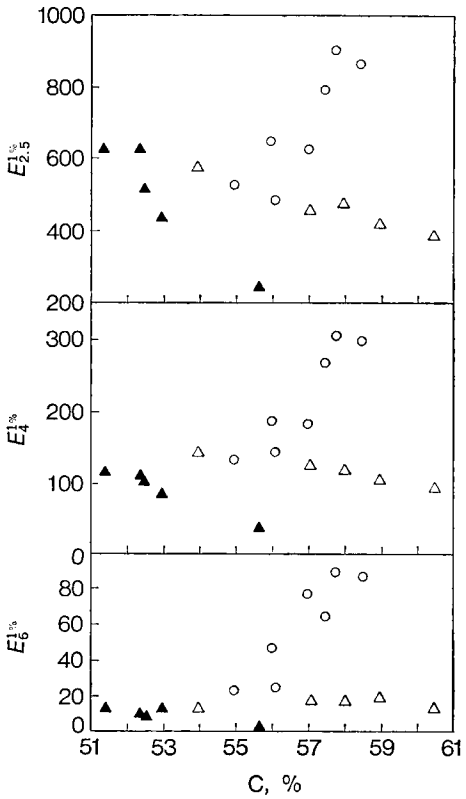


Fig. 3-5. $E^{1\%}$ *vs.* C% diagrams. ○: HA; △: FA (group 1); ▲: FA (group 2).

FAs were divided into two groups: Group 1, #5 FA–#6 FA_1–#3 FA_1–#4 FA_1–#4 FA_2 and group 2, #1 OH FA–#1 P FA–#2 FA–#6 FA_2–#3 FA_2. Correlation coefficients between the $E_6{}^{1\%}$, $E_4{}^{1\%}$ or $E_{2.5}{}^{1\%}$ values and carbon content for each group were respectively 0.23, −0.98 and −0.96; and −0.85, −0.99, and −0.95. Thus, high negative correlations were found except between the $E_6{}^{1\%}$ value and carbon content for group 1, indicating that the light absorbability of FAs in each group decreases with the increase of carbon content.

As seen in Table 3-2, the increase of the carbon content in each group coincided with the increase of hydrogen and nitrogen content and the lowering of C/H and C/N ratios.

The correlation coefficients between $E_6{}^{1\%}$, $E_4{}^{1\%}$, or $E_{2.5}{}^{1\%}$ and E_4/E_6 were: group 1, −0.71, 0.81 and 0.86; group 2 ,−0.43, 0.25 and 0.29. Thus,

the correlation between the E values and E_4/E_6 values seems insignificant or altogether lacking.

In accordance with these findings, all the parameters adopted may be assumed useful for characterizing the absorption spectra of soil HAs and FAs.

It is evident, then, that absorption spectra do contribute to the differentiation of soil HAs and FAs. Thus, it is desirable to establish a standard method for the determination of absorption spectra, including suitable parameters for characterizing them.

The present study confirmed the existence of correlations between their spectral characteristics and elementary composition. The spectral characteristics differed notably between HAs and FAs, suggesting that distinct differences exist in their chemical configurations. In addition, there were regularities in the elementary composition of HAs and FAs studied here which will be described further in Chapter 5. Regrettably, the number of samples dealt with is rather limited, and the validities of the correlations and regularities recognized here should be examined further in future investigations.

3.2.3. Ionization- and reduction-difference spectra

Differential spectral techniques have been widely used in lignin chemistry (Terashima, 1982). There have been several reports (Kobo and Fujisawa, 1962; Suzuki and Kumada, 1972; and Tsutsuki and Kuwatsuka, 1979d, e) on the difference spectra of HAs, which suggest that difference spectra are useful for discriminating individual HAs from one another. To confirm this, the ionization difference (ΔE_i) and reduction difference (ΔE_r) spectra of the HAs and FAs used in the previous paragraph were determined (Kumada, 1985b).

In the present study, the ΔE_i and ΔE_r spectra refer to the difference spectra between the pH 13 and pH 6 solutions and between the pH 10 and $NaBH_4$-treated pH 10 solutions, respectively.

Determination of ΔE_i spectra

Sample solutions containing 5 to 40 mg of HA or FA in 100 ml of 0.1 N NaOH were prepared according to the procedure described previously (Section 3.2.1.). Ten ml of the sample solution was pipetted into a 25 ml volumetric flask, and the flask was filled up to volume with 0.1 N NaOH. This solution was designated as pH 13 solution. Another 10 ml aliquots of the sample solution were adjusted to pH 6.0 with diluted phosphoric acid (1:9), and the pH 6 solution prepared by adding phosphoric acid to 0.1 N NaOH was added to make a total volume of 25 ml.

The ΔE_i spectra from 230 to 700 nm were obtained by determining the difference in the optical density between the pH 13 and pH 6 solutions. The

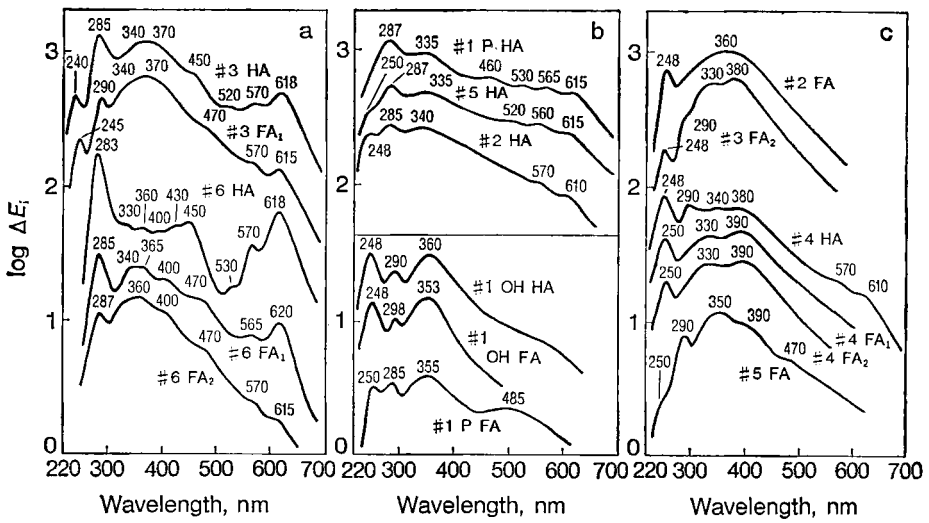


Fig. 3-6. ΔE_i spectra. a: group 1; b: upper, group 2; lower, group 3; c: group 4.

values obtained were plotted in the form of log ΔE_i *vs.* λ curves. $\Delta E_1{}^{1\%}$ values at selected wavelengths were calculated, where $\Delta E_i{}^{1\%}$ was the difference in the optical density of the solution containing 1% HA- and FA carbon. The determination was conducted immediately after the preparation of sample solutions. Figure 3-6 shows the ΔE_i spectra of samples used.

First, absorption bands in the visible region will be dealt with. In the absorption curves of samples #3 HA and FA_1 and #6 HA, FA_1 and FA_2, the absorption bands at around 615, 565, and 450 nm, corresponding to Pg absorption, were more distinct than in the ordinary absorption curves. Additionally, the bands at 530 and 430 nm in #6 HA and a band at 520 nm in #3 HA were also attributed to Pg absorption, because the Pg fractions (G_1, G_2, and G_3) separated from soil (Kumada and Sato, 1980) also showed these bands.

Pg absorption was observed in the ΔE_i spectra of samples #1 P, #2, #4, and #5 HAs which did not appear in the ordinary absorption spectra, as was previously noted by Tsutsuki and Kuwatsuka (1979a).

Sato (1974) prepared alkaline solutions containing a purified Pg fraction and various HAs showing no Pg absorption, and determined their absorption spectra. In one Rp type HA having a high E_4/E_6 ratio, Pg absorption was detected when the Pg content was 2%. In an A type HA with a low E_4/E_6 ratio, however, shoulder-like absorption bands were observed only when the Pg content exceeded 6%. This finding suggests that Pg absorption

can be observed only when the Pg content exceeds a certain level, and the lower the E_4/E_6 ratio of HA, the higher is the level necessary for the Pg fraction to be detected. This is the limitation of the method proposed by Sato (1974) for estimating Pg content, as he himself pointed out. In any case, the fact that the ΔE_1 spectra of the HAs having lower E_4/E_6 values showed Pg absorption can be explained on the basis of the experimental results obtained by Sato. It is nevertheless worth noting that 6 out of 7 HAs and 3 out of 10 FAs showed Pg absorption in the ΔE_1 spectra. This finding suggests the universal existence of the Pg fraction in soil HAs and FAs.

Other than the samples mentioned above, #1 P FA and #5 FA each showed an absorption band in the visible region, near 485 and 470 nm respectively, suggesting the presence of special kinds of dissociated groups.

In the UV region, there were a number of absorption bands, but most of them were broad and overlapped with each other. Thus, it was rather difficult to determine exactly the positions of peaks and shoulders.

In sample #6 HA, absorption bands were observed at 283, 330, and 400 nm, and the band at 283 nm was the strongest among them. It is not certain whether all of these bands are due to the dissociation of the DHPQ moiety. In general, the absorption bands found in the UV region of the ΔE_1 spectra of samples showing Pg absorption in the visible region may be due to the dissociation of the DHPQ moiety and other groups, and it is at present hardly possible to distinguish them from one another.

With respect to the ΔE_1 spectra, the samples were tentatively divided into the following four groups.

Group 1: Samples #6 HA, FA_1 and FA_2 and #3 HA and FA_1. These are characterized by the presence of bands at 285 and 365 nm as well as Pg absorption in the visible region of ΔE_1 spectra and in ordinary absorption spectra.

Group 2: Samples #1 P, #5 and #2 HAs. These showed Pg absorption in the visible region of ΔE_1 spectra and had three bands at 285, 335, and 245 nm. The ΔE_1 values of these bands decreased in this order, and #1 P HA did not show the band at 245 nm.

Group 3: Samples #1 OH HA and FA and #1 P FA. These had fairly distinct absorption bands near 250, 290, and 355 nm. The shapes of their ΔE_1 spectra were similar to those of lignin and Rp type HAs reported by Kobo and Fujisawa (1962) and Tsutsuki and Kuwatsuka (1979d).

Group 4: The rest of the samples, #2 FA, #3 FA_2, #4 HA, FA_1 and FA_2 and #5 FA, were included in this group.

Sample #4 HA showed Pg absorption in the ΔE_1 spectrum, but its $\Delta E_1^{1\%}$ value at 615 mn as well as $E_6^{1\%}$ value was small, and the shape of its ΔE_1

spectrum in the UV region was different from those of the HAs belonging to group 2. The ΔE_1 spectrum of this HA may be described as a composite of those of #4 FA_1 and the Pg fraction. With the exception of #4 HA, the samples belonging to group 4 were all FAs, and had a common absorption band at 245 nm as well as two or three bands in the region of 290 to 390 nm. At present, it is difficult to characterize them further.

Tsutsuki and Kuwatsuka (1979d) demonstrated that the ΔE_1 spectra of A, B, Rp, and P type HAs were different from one another. Group 1, 2, and 3 described above may correspond to P type, A type and Rp type, respectively. Sample #4 HA in group 4 may correspond to B type. Out of the 7 HAs studied by Kobo and Fujisawa (1962), numbers 5 and 2 seem to correspond to group 3 and 2, respectively.

In lignin chemistry, ΔE_1 spectra are interpreted in terms of various kinds of phenolic hydroxyl groups present. As discussed at length by Tsutsuki and Kuwatsuka (1979d), the ΔE_1 spectra of the HAs belonging to group 3 and Rp type may be interpreted in terms of lignin chemistry to a certain extent, but there exist considerable differences in the ΔE_1 spectra between lignin and the HAs mentioned above. The ΔE_1 spectra of other HAs are remarkably different from that of lignin. Therefore, interpretation of the ΔE_1 spectra should be left to future investigation.

ΔE_r spectra

Determination of ΔE_r spectra Ten ml aliquots of the sample solution were placed in two beakers, and the pH was adjusted to 10.0 with phosphoric acid. After the addition of solid $NaBH_4$ corresponding to about ten times the amount of the sample weight to one of the beakers, the volume of both solutions was adjusted to 25 ml with the pH 10 solution prepared by adding phosphoric acid to 0.1 N NaOH.

The ΔE_r spectra from 230 to 700 nm were obtained by determining the difference in the optical density between the pH 10 solution and $NaBH_4$-treated pH 10 solution 24 hr after the addition of $NaBH_4$. The values were plotted in the form of log ΔE_r *vs.* λ curves.

The ΔE_r spectra of the samples except #1 OH FA are shown in Fig. 3-7. As mentioned above, ΔE_1 spectra enabled us to divide the samples into four groups. Accordingly, it may be reasonable to examine whether this grouping is also applicable to ΔE_r spectra.

Group 1: In the visible region, samples #6 HA and FA_1 and #3 HA showed two absorption bands near 460 and 570 nm. These bands correspond to the shoulder-like bands observed in the ordinary absorption spectra of #6 HA determined at pH 10 in the previous paragraph; thus they are attrib-

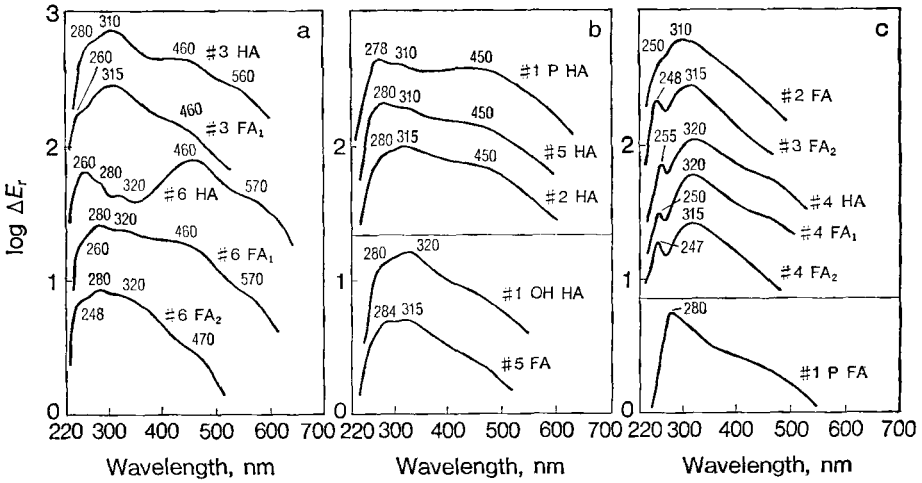


Fig. 3-7. ΔE_r spectra. a: group 1; b: upper, group 2; lower, group 3; c: upper, group 4; lower, exception.

uted to Pg absorption. In samples #6 FA_2 and #3 FA_1, only a weak shoulder band near 460 nm was observed, probably due to the low Pg content.

In the UV region, samples #6 HA, FA_1 and FA_2 showed three bands at 250, 280, and 320 nm, but for samples #3 HA and FA_1, two bands were observed near 310 and 280 or 260 nm. Judging from the shapes of the ΔE_r spectra, the third band in #3 HA and FA_1 might have overlapped with the adjacent bands.

Group 2: Samples #1 P, #2 and #5 HAs belonged to this group. The $\Delta E_r^{1\%}$ values at 280 and 315 nm were large. The curves were very broad, and the plateau-like absorption extended to 450 nm and beyond, judging from Fig. 3-7. It is assumed that the HAs belonging to this group contain some carbonyl groups which affect light absorption throughout the whole UV and visible region. The Pg fraction revealed in the ΔE_1 spectra may partly account for the strong light absorption in the visible region.

Group 3: Samples belonging to this group were characterized by lignin-like ΔE_1 spectra. The ΔE_r spectrum of sample #1 OH HA was very broad and consisted of a peak at 320 nm and a shoulder near 280 nm. Sample #5 FA may be included in this group, because the shape of the ΔE_r spectrum was similar to that of #1 OH HA. But #1 P FA should be excluded from this group, because it showed only one peak at 280 nm.

Group 4: Except for #5 FA, the samples belonging to this group, #2 FA, #3 FA_2, #4 HA, FA_1 and FA_2, were characterized by two absorption bands near 315 and 250 nm, and the former was stronger than the latter.

Thus, groupings based on ΔE_i and ΔE_r spectra were almost the same, except that with respect to ΔE_r spectra sample #5 FA moved from group 4 to group 3, and #1 P FA was excluded from all groups.

The following can also be pointed out. (1) The HAs and FAs showing Pg absorption belonged to group 1. (2) Samples #1 P, #2 and #5 HAs having larger $E_6^{1\%}$ values and lower E_4/E_6 ratios constituted a group in which the FAs obtained from the same soils were not included. On the other hand, #1 OH and #4 HAs having smaller $E_6^{1\%}$ values and higher E_4/E_6 ratios belonged to the same groups as the FAs obtained from the same soils, suggesting structural similarities between the HAs with a lower degree of humification and the FAs.

The results obtained in the present study indicate that ΔE_r spectra of HAs and FAs are characterized by the presence of absorption bands at 315 nm as well as at 250 and/or 280 nm, and in some HAs, the strong light absorption extended to the visible region. Even if it is almost certain that these bands result from various kinds of carbonyl groups, their chemical structures should be elucidated in the future.

Contribution of ΔE_i and ΔE_r to E

HAs and FAs are characterized by their brown color, *i.e.*, appreciable light absorption in the visible region which increases with decreasing wavelength. But we know little about the mechanisms of light absorption. Absorption spectroscopy is expected to serve as a means of solving this problem.

It has been said in lignin chemistry that ΔE_i and ΔE_r spectra are respectively associated with the presence of various kinds of phenolic hydroxyl and carbonyl groups (Terashima, 1982). Although it remains uncertain to what extent this interpretation is applicable to HAs and FAs, the present study confirmed that their light absorption in the visible region was reduced considerably by lowering the pH of a solution and adding $NaBH_4$ to the solution.

To estimate the quantitative contribution of ΔE_i and ΔE_r spectra to ordinary absorption spectra, the ratios of ΔE_i and ΔE_r values to E values at 400, 500 and 600 nm were calculated (Table 3-3). (1) In most cases, the $\Delta E_r/E$ values were higher than the $\Delta E_i/E$ values at each wavelength. (2) Both values tended to increase with the increase in wavelength. (3) The sum of $\Delta E_i/E$ and $\Delta E_r/E$ values at 400 nm ranged from 37 to 63% for HAs and 49 to 78% for FAs. The sum of values at 500 and 600 nm was either nearly equal or the latter was higher than the former. The sum of $\Delta E_i/E$ and $\Delta E_r/E$ at 600 nm ranged from 60 to 93% for HAs, and from 82 to 105% for FAs. Thus, a large part of the light absorption in the visible region of HAs and FAs can be attributed to ΔE_i and ΔE_r.

TABLE 3-3
Contribution of ΔE_i and ΔE_r to E in the visible region.

Sample			400 nm	500 nm	600 nm	Sample			400 nm	500 nm	600 nm
#1 OH	HA	I[a]	22	24	34	#4	HA	I	30	32	43
		II	28	41	39			II	33	49	45
		III	50	65	73			III	63	81	88
#1 P	HA	I	14	20	32		FA_1	I	30	33	39
		II	27	44	41			II	38	58	55
		III	41	64	73			III	68	91	94
	FA	I	17	28	42		FA_2	I	32	36	40
		II	32	47	40			II	46	62	60
		III	49	75	82			III	78	98	100
#2	HA	I	19	25	33	#5	HA	I	15	20	30
		II	24	36	35			II	22	31	30
		III	43	61	68			III	37	51	60
	FA	I	30	34	39		FA	I	26	32	42
		II	40	53	45			II	33	44	43
		III	70	87	84			III	59	76	85
#3	HA	I	27	21	40	#6	HA	I	20	13	46
		II	23	37	34			II	31	60	47
		III	50	58	74			III	51	93	93
	FA_1	I	32	32	46		FA_1	I	25	29	47
		II	38	57	56			II	35	51	49
		III	60	89	102			III	60	90	96
	FA_2	I	35	34	39		FA_2	I	35	42	56
		II	43	60	55			II	40	51	59
		III	78	94	94			III	75	93	105

[a] I, $\Delta E_i/E$; II, $\Delta E_r/E$; III, I+II (%).

The DHPQ structure observed in 9 out of 17 samples may be responsible for both the ΔE_i and ΔE_r. In HAs with high $E_6^{1\%}$ values, *i.e.*, samples #1 P, #2 and #5 HAs, light absorption unrelated to ΔE_i and ΔE_r should not be overlooked. This may be partly attributable to a turbostratic structure (see Section 10.1.).

The results obtained in the present study clearly show that ΔE_i and ΔE_r spectra are not only useful for differentiating individual soil HAs and FAs, but also helpful in explaining their dark color, *i.e.*, specific light absorption in the visible region. Furthermore, the results tell us that the absorption spectra of HAs and FAs are never featureless. Further investigation is necessary to interpret the diverse absorption bands in these spectra.

3.3. Speculation on the Mechanisms of Light Absorption of Humic Acids

As mentioned above, the sum of $\Delta E_1/E$ and $\Delta E_r/E$ values at 600 nm for HAs ranged from 60 to 93%, suggesting that more than half to almost light absorption can be explained by these spectra. However, this calculation premises that additivity holds between ΔE_1 and ΔE_r values, and this premise should be verified.

Tsutsuki and Kuwatsuka (1979e) studied extensively not only ΔE_1 (pH 12.4–7) and ΔE_r ($NaBH_4$) spectra but also ΔE_1 (pH 7–3.3) and ΔE_r (reduction with $Na_2S_2O_4$ in 0.1 N NaOH or phosphate buffer at pH 7) spectra, and concluded that quinone and the other carbonyl groups, phenolic hydroxyl group, and carboxyl group participate in the light absorption of HAs in the visible region, and that the degree of participation of these functional groups varies with the type of HAs.

It is well known that HAs contain a relatively high concentration of free radicals, and these free radicals may contribute to their light absorption. As postulated by Ziechmann (1972, 1977) and Lindquist (1982), inter- and intramolecular charge transfer spectra may also contribute to light absorption; the mechanisms of these factors seem to deserve further study.

As discussed earlier, Pg absorption due to DHPQ moiety plays an unexpectedly important role in light absorption in the visible region. If Pg absorption is excluded, however, UV and visible spectra of HAs are almost featureless, and analogous to one another. In this connection, the author (1965) considered that the similarity of their spectra suggests that we are dealing with compounds with similar basic structures. On the other hand, Hayes and Swift (1978) and Stevenson (1982) considered that the UV- and visible spectra of humic substances result from the overlap of the absorbances of various chromophores. Neither hypothesis, however, explains the peculiar optical properties of HAs pointed out in Section 2.1, that is: (1) light absorption decreases with increasing wavelength, and the log E *vs.* λ curve is almost straight, and (2) the larger the light absorption of an HA, the lower is the inclination of the curve to the axis of wavelength, so that the curves rarely cross each other.

Why do the absorption spectra of HAs exhibit such highly conformable shapes? In the author's opinion, this question is almost the same as the question of what humic substances are, because brown color is one of the essential attributes of HAs, probably the most important. In other words, humic substances are nothing but the chemical configurations which make up the brown color.

The author earlier postulated that the dark (brown) color of HAs is principally due to the existence of numerous varied kinds of conjugated double bonds which are primarily distributed randomly in the HA molecules, and that conjugated double bond systems should be regarded as the nature of humic substances (Kumada, 1965). Humification would then mean the formation and development of the randomly distributed, conjugated double bond systems in the HA molecules. This system may be formed by the oxidative polycondensation of polyphenols, and also by the oxidative disintegration of high molecular polymers, especially lignins. These processes may correspond to the polyphenol and lignin theories, respectively, after Stevenson (1982). It is also inferred that both processes proceed abiotically, even if the catalytic action of soil inorganic constituents and enzymes is considered, and therefore the formation of the conjugated double bonds occurs randomly. Based on the evidence obtained by X-ray diffraction analysis (see Chapter 10), these processes might be regarded as an early stage of carbonization of organic materials taking place in soil, under the normal temperature and pressure and in the presence of oxygen and moisture.

These ideas on humic substances and humification can be further expanded as follows: The conjugated double bond systems are composed of various cyclic and linear carbon to carbon double bonds $(C=C)_n$, (n: degree of conjugation), quinone and other carbonyl groups, stable free radicals, inter- and intramolecular charge transfer complexes *etc.*, and these form hybridized π orbitals. With increasing pH of the medium, phenolate and carboxylate anions may participate in the π orbitals.

If humic substances in HA are the conjugated double bond systems mentioned above, these HAs are thought to belong to a sort of organic semi-conductor (Mikawa and Kusabayashi, 1977), and have an energy band structure, as is the case for carbonaceous materials (Mizushima and Okada, 1970; Ohtani and Sanada, 1980; Inagaki, 1985).

Mizushima and Okada (1970) stated: "In the course of carbonization of organic materials, their polycondensation and thermal decomposition proceed, resulting in the gradual approach of valence electron band and conduction band, and the shifting of optical absorption edge from the UV region to the visible region. As a result, the color of the originally achromatic materials changes from yellow to brown and finally to black when absorption edge shifts to the deep infrared region."

It is presumed that in the case of humification of SOM too, oxidative depolymerization or polycondensation causes a change similar to this early stage of carbonization. In the progress of humification, valence electron band and conduction band in the HA molecule would gradually approach each other because of the increase of the density of π electron cloud in the

hybridized π orbitals. Thus, regular transitional changes in the absorption spectra of HAs with the progress of humification may be explained by the energy band theory.

These considerations constitute an extremely primitive working hypothesis on the color of HA and the nature of humic substances. The validity of the hypothesis remains to be verified. The idea that humic substances are conjugated double bond systems in HA can also be applied to FA, especially its PVP adsorbed fraction.

Certain points are worthy of emphasis: (i) Humification is an early stage of carbonization at normal temperature and pressure in the presence of oxygen and moisture. (ii) Humic substances are simply various conjugated double bond systems randomly distributed in the HA molecules which are responsible for the brown color. (iii) Experimental and theoretical studies on the mechanisms of light absorption are essential in humus chemistry. (iv) the introduction of methods of research or methodology in the field of carbonization engineering as well as coal science appears advantageous for the furthering of humus chemistry.

According to Orlov (1985), Pavel and Zazvorka (1965) considered that the absorption spectra of HAs in the 400–750 nm range can be expressed,

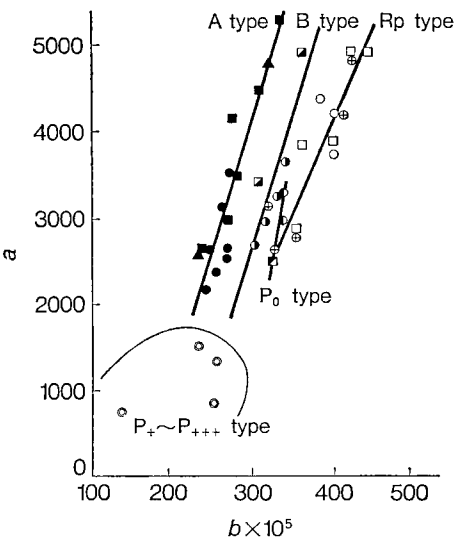


Fig. 3-8. Relationships between constants a and b in the equation $E=a\cdot 10^{-b\lambda}$. Linear regression and correlation coefficient: A type: $a=3.00\times10^6b-4876$, $\gamma=0.918$***; B type: $a=3.01\times10^6b-6396$, $\gamma=0.895$**; Rp type: $a=2.13\times10^6b-4455$, $\gamma=0.878$***; P_0 type: $a=5.12\times10^6b-14196$, $\gamma=0.921$*.

in the first approximation, as an exponential function of wavelength, and proposed the equation.

$$D_\lambda = A' \cdot e^{-a'\lambda}$$

where D_λ is the optical density at the wavelength λ, and A' and a' are constants.

Similarly, but independently, Tsutsuki and Kumada (1980) proposed the same equation

$$E = a \cdot 10^{-b\lambda}$$

where E is the optical density at the wavelength λ, and a and b are constants, to express the absorption spectra of HAs in the visible region. By taking the log of these values, we get

$$\log E = \log a - b\lambda.$$

This equation indicates that the logarithm of optical density decreases proportionately to wavelength.

Thirty eight of the HAs listed in Table 5-1 were used by Kuwatsuka *et al.* (1978) and their $E_6^{1\%}$ and $\Delta\log K$ values determined. Using these values, constants a and b were calculated and plotted on an a *vs.* b diagram as shown in Fig. 3-8. As seen, the values are widely distributed, indicating that constants a and b vary with individual HA samples. Significant correlations are noted, however, between the a and b values of each type of HAs, except those of P type. Interpretation of these observed correlations remains to be made in future.

Chapter 4

P Type Humic Acid

In the classification system of HAs proposed by the author, P type is a group which has Pg absorption. This type was first found in Podzols and thought to have limited distribution. Now, however, it is recognized as being distributed worldwide.

P type is worthy of note for several reasons: (i) In soils having P type HAs, FAs may contain the Pg fraction, as observed by Schnitzer and Skinner (1968) and Kumada (1985a). (ii) It was illustrated that the HAs with no Pg absorption in the ordinary absorption spectra can reveal it in the ΔE_1 spectra (Section 3.2.). (iii) The adjacent OH and O in the DHPQ nucleus of P type may serve as the ligand for polyvalent cations to form complexes and participate in their translocation (leaching and deposition) in soils, because Nakabayashi *et al.* (1982) reported that the Pg fraction was stabilized by combining with iron in a Brown forest soil. (iv) The DHPQ may take place in the constitution of the turbostratic structure characteristic of A type HA. (v) The DHPQ nucleus may be a precursor of perylene, one of the most abundant condensed aromatic rings observed in the sediment of the sea bottom. Items (iv) and (v) are merely suppositions; if confirmed, the significance of the P type would be remarkably increased.

In this chapter, information on the distribution, fractionation, sources, and chemistry of the P type are reviewed, and quinone pigments found in soils are described.

4.1. Distribution of P Type Humic Acid

Most HAs of the Podzols, Brown forest soils, Red and Yellow soils, Alpine grassland soils, and Alpine meadow soils in Japan belong to P type (see also

Chapter 6). The type is comparatively rare in the A horizon of Black soils, but rather abundant in their B and C horizons, and quite rare in peat soils and paddy soils. These findings were supported by comprehensive studies on the humus of forest soils conducted by Kawada (1972, 1975a, b, 1976; Kawada and Kojima, 1976).

Orlov (1968) observed a wide distribution of P type in the various soils of the Soviet Union, *e.g.*, Sod-podzolic soils, Brown soils, Grey forest soils, Yellow soils, Brown forest soils, Mountain meadow soils,but did not observe this type in Chernozems, Chestnut soils, Gray soils or alluvial soils. P type was also found by Kumada and Hurst (1967) in soil samples from the United States and most of the countries in Western Europe, by Lowe and Tsang (1970) in Canada, by Ohsumi and Ohta (1975) and Ohta (1985) in Nepal, by Kumada in Taiwan (unpublished data), and by Sato in the Federation of Malaysia and Indonesia (unpublished data). The frequency of P type HAs in other countries such as the soils of Great Britain, Czechoslovakia, Canada, and Thailand will be discussed in Chapter 6. The worldwide distribution of P type in forest soils and some grassland soils has thus been well established.

4.2. Fractionation of P Type Humic Acid

Since P type was first observed, its specific absorption bands were supposed to be due to the presence of a chromophore; trials to separate it, however, were unsuccessful for several years. In 1961, Sato noticed that when one drop of an alkaline solution of P type was put on filter paper, a circular diagram composed of double rings was formed; the outer ring was reddish brown in appearance, and the inner a dirty green. From this fact, it was presumed that the P type was composed of at least two fractions which were easily separable. This was confirmed by column chromatography using cellulose powder or Sephadex as adsorbent, and a mixture of EtOH and alkali or alkali as eluent (Kumada and Sato, 1962).

An example of this chromatography follows: A cellulose (Whatman) column (1×13 cm) was prepared, and a small amount of P type HA dissolved in 0.1% NaOH was mounted on the top of the column and developed by EtOH-0.1% NaOH (2:1). A brown band descended rapidly, and a green band remained on the upper layer of the column. The brown band was eluted easily (fraction 1), but the latter reddish brown effluent fraction of this band showed a green tint, and was fractionated separately (fraction 2). The green band was eluted by 0.1% NaOH and a green solution was obtained (fraction 3).

By adding HCl to fractions 1 and 2, reddish brown precipitates were formed readily, as was the case with ordinary HAs. Fraction 3 first changed

to pinkish red, and then a reddish brown gel was gradually formed after standing. It was noticed that the green solution was faintly red in reflected light, indicating the existence of dichroism. The three fractions were also obtained by using Sephadex G-25 as adsorbent and 0.1% NaOH as eluent.

Absorption spectra of fraction 3 showed three distinct peaks at 617, 570, and 450 nm, those of fraction 2 were similar but broader, and those of fraction 1 were far broader but still preserved shoulder-like absorption bands near 615 and 450 nm.

These results showed that P type HA could be separated into Pg (green fraction), Pb (brown fraction), and an intermediate dark green fraction by column chromatography using cellulose powder or Sephadex. When this experiment was carried out, the Pg fraction was designated as green HA, but "green" HA was incorrect, because HA should be brown in color according to the definition.

The green fraction (Pg) may be regarded as a fairly common impurity co-extracted with a brown HA. On the other hand, it is certain that part of the Pg remains in the Pb and the intermediate fractions. In these fractions, the Pg may be merely an admixture, or may be firmly associated with the

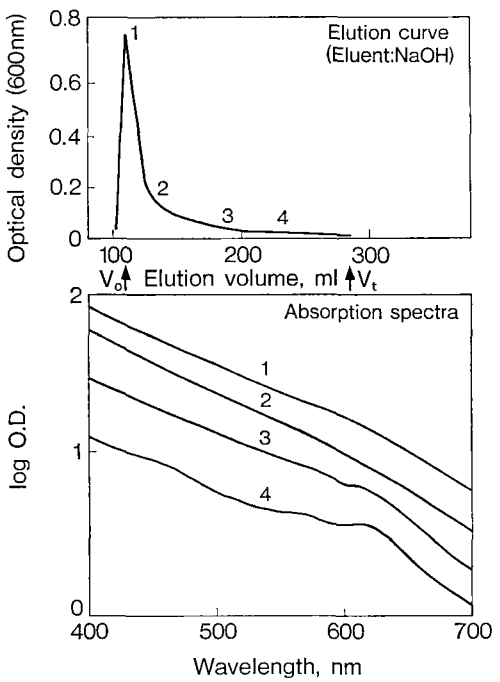


Fig. 4-1. Fractionation of Kuragari humic acid.

brown fractions. To throw light on these questions, gel chromatography of several HAs was done, and certain characteristics of the Pg were investigated (Kumada and Sato, 1980). Part of the results are outlined below:

Here, three HA samples are exemplified, Tsubame and Tateyama HAs belonging to the P_{+++} and P_{+} types, respectively, and Kuragari HA to the B type.

Gel chromatography Gel material, Sephadex G-50; eluent, 0.1 N NaOH; column size, 2.5 × 60 cm; amount of HA, 7–35 mg.

Column effluents were collected in 5 to 15 ml fractions with a fraction collector, and elution curves were drawn based on the optical density at 600 nm in a 10 mm cell. The absorption spectrum of each effluent fraction was determined within the range of 400 or 220 to 700 nm. When the optical density of an effluent fraction was too low to determine the absorption spectrum, neighboring fractions were appropriately combined. In these figures, the number above each absorption spectrum identifies the relevant fraction(s) in the elution curve.

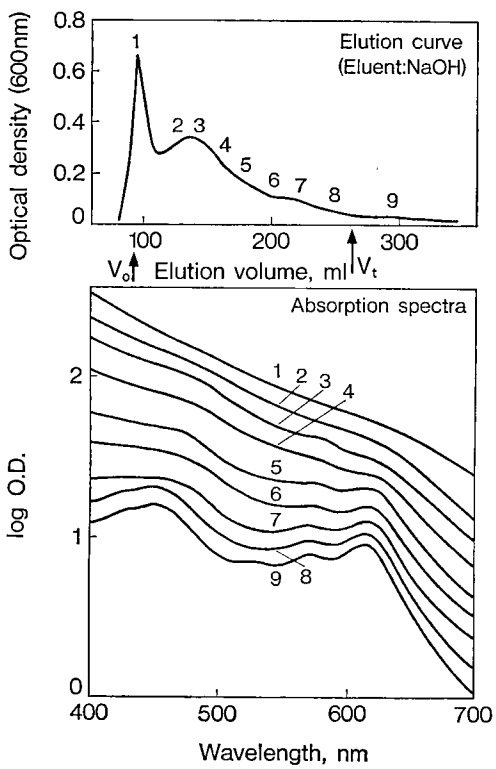


Fig. 4-2. Fractionation of Tateyama humic acid.

In Kuragari HA which has no Pg absorption (Fig. 4-1), a large part of the sample was excluded, and the tailing part was eluted within V_t. Absorption spectra of Nos. 3 and 4 showed a faint, and a fairly distinct Pg absorption, respectively. As illustrated here, even if an HA has no Pg absorption as a whole, it is not uncommon for Pg absorption to be apparent in the lower molecular fractions.

The elution curve of Tayetama P_+ type HA (Fig. 4-2) exhibited a sharp peak at V_0, two peaks within V_t and a weak tailing part after V_t. The intensity of Pg absorption increases with increasing fraction number, *i.e.*, probably with decreasing molecular weight.

A more complicated elution curve (Fig. 4-3) was obtained for Tsubame HA; it showed the strongest Pg absorption among the samples. The curve exhibited a sharp peak at V_0, three peaks within V_t and a small peak after V_t.

As shown in Fig. 4-3, the effluent fractions of Tsubame HA could be divided into six groups: B_1, B_2, BG, G_1, G_2 and G_3. Fractions belonging to each particular group were combined, and acid precipitable materials were recovered. The weight distribution of the six fraction groups was 100, 58, 32, 22, 8, and 5 mg, respectively. In an alkaline solution, the color of B_1 and B_2 was dark brown; that of BG, greenish brown; and that of G_1, G_2, and G_3, green. Figure 4-4 shows absorption spectra for these groups. The intensity of Pg absorption increased with increasing fraction number. The λ_{max} values for G_3 were situated at 280, 445, 568, and 611 nm. It should be noted that even the B_1 and B_2 groups had distinct Pg absorption, although the intensity was weak. Attempts to remove this absorption by repeated gel chromatography proved unsuccessful.

Table 4-1 gives the elementary composition of Tsubame HA and its G_1 and G_2 fraction groups. The C, H, and N contents of the original HA were similar to those of P and Rp(2) type HAs given by Kuwatsuka *et al.* (1978), but the G_1 and G_2 as well as Tsubame Pg reported by the same authors

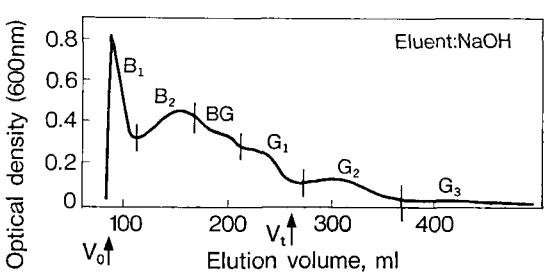


Fig. 4-3. Elution curve of Tsubame humic acid.

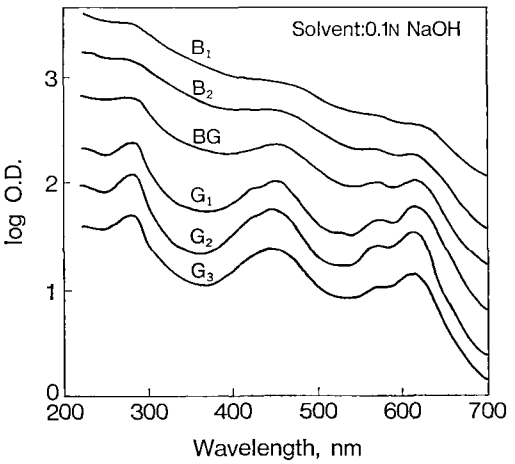


Fig. 4-4. Absorption spectra of the Tsubame fraction groups.

TABLE 4-1
Elementary composition of Tsubame humic acid and its G_1 and G_2 fraction groups.

	C	H	N	O
Unfractionated	37.91	42.74	1.23	18.12
G_1	47.98	28.30	0.36	23.36
G_2	50.06	27.79	0.29	21.86

Figures expressed as atomic number ratios on moisture- and ash-free bases.

revealed the lowest H and N contents among the 38 HA samples which they examined. The G_1 and G_2 fraction groups were characterized by very high C/H and C/N ratios.

The results for ultrafiltration, TLC and gel chromatography of the G group (G_1, G_2, and G_3) may be summarized as follows:

(1) G group dissolved in alkali (pH 8.5–11) was not passed through an ultramembrane (M.W. 1,000).

(2) Part of the G group was extracted with ethyl acetate from acidified solutions. TLC of the ethyl acetate extracts gave one or two main spots as well as diffuse bands, and these exhibited orange fluorescence under ultraviolet light. The materials remaining in the acid solutions after ethyl acetate extraction were green in alkaline solution, and had distinct Pg absorption.

(3) G group was irreversibly adsorbed on Sephadex G-10 when 0.1 N NaOH was used as eluent. When 0.1 N NaOH containing 1.5 M urea, recommended by Yonebayashi (1976) as being suitable for fractionation based on molecular weight differences, was used on the same gel, a large part of each

G fraction was excluded, a small part gradually eluted, and irreversible adsorption was observed.

From these experimental results, it seems reasonable to conclude that the G group is composed mainly of relatively lower molecular weight, heterogeneous polymers containing DHPQ nucleus as their indigenous monomer.

The findings also suggest that B_1 and B_2 groups are composed mainly of HAs in which the G group is incorporated to some extent. The BG group appears to be a mixture of the B_2 and G_1 groups. This idea may be applicable to all P type HAs, although the quantity of the G group and the extent of its incorporation in HAs varies remarkably with each P type.

No conclusion can yet be drawn on the form of existence of the G group in the B_1, B_2 and BG groups. There are two possibilities: the former may be mixed or may be inseparable from the latter. The fact that attempts to remove Pg absorption by repeated gel chromatography were unsuccessful suggests the second possibility; but a negative result cannot serve as proof. Perhaps a third possibility should be considered that there are both forms of existence.

4.3. Origin of Pg

When the green fraction was first separated from the remaining P type by chromatography, it was assumed that it might be a derivative of porphyrins, because several metal-porphyrins such as coproporphyrin and mesoporphyrin (Schwartz *et al.*, 1960) had similar absorption maxima to the Pg. This assumption was denied when the authors demonstrated the existence of DHPQ nucleus in the Pg (Sato and Kumada, 1967). Before this, Kumada and Sato (1965) found that numerous blackish brown granules (*ca.* 1 mm in diameter) existed in the H horizon of an Alpine podzol, and the absorption spectrum of the HA-like fraction of the granules almost coincided with that of the P type. Extensive survey revealed that this kind of granules was widely distributed not only in Podzols but also in soils having P type HA. Consequently, it was inferred that the granule was one source of the Pg.

In 1967, the author observed the wide distribution of these P type HAs and the black granules in the Podzols, Brown earths and Alpine grassland soils of Great Britain (Kumada and Hurst, 1967). Professor Alan Burges, at that time in Liverpool University, identified the granules as sclerotia of fungi.

Soil horizons which show clear evidence of P type commonly contain small spherical black fungal sclerotia with a mean diameter of 0.5 ± 0.4 mm (British) to 1.1 ± 0.4 mm (Japanese and Swedish). These sclerotia have a brittle outer shell. Most of them are hollow, but some have a characteristic

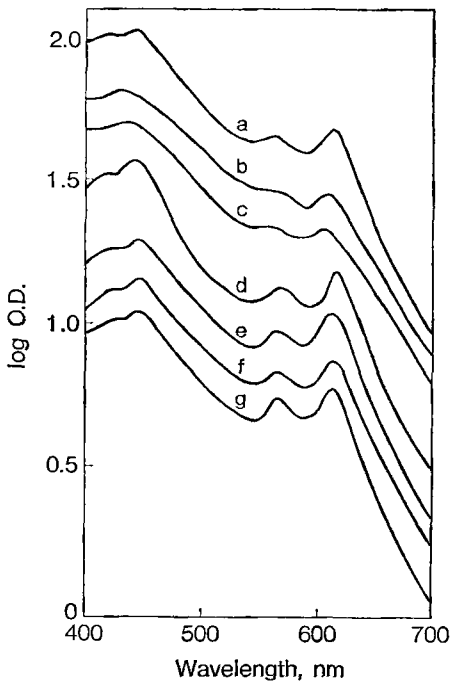


Fig. 4-5. Absorption spectra of the Pg fraction. Isolated from soil: (a) Delamere; (b) Tyrebagger; (c) Burton Wood. Isolated from fungal sclerotia: (d) Delamere; (e) Tyrebagger; (f) Burton Wood; (g) Norikura (Japan).

internal structure in transverse section and can easily be recognized in thin sections of resin impregnated soils. Their appearance fits descriptions given for sclerotia of the organism *Cenococcum graniforme* (Ferdinandsen and Winge, 1925) which has a worldwide distribution in temperate and arctic-alpine climatic zones, and which forms ectotrophic and ectendotropic association with unusually large numbers of tree, shrub, and herbaceous genera.

The absorption spectra of the Pg isolated from different soils and from fungal sclerotia sampled in Great Britain are shown in Fig. 4-5. The Pg fractions were isolated by gel filtration through columns of Sephadex G-25 from precipitates by acidification of alkaline extracts. The similarity of the absorption spectra of the Pg from soils and fungal sclerotia is quite evident. All of them have λ_{max} near 615, 570, 450, and 430 (infl.) nm.

Green pigments having almost the same absorption spectra as the Pg were obtained from cultured mycelia of *Cenococcum graniforme* and *Altenaria tenuis* by Vol'nova and Mirchink (1972), suggesting that the Pg is produced by these fungi. Sato (1976b) obtained Pg-like substances from the fruiting

bodies of several wood-rotting fungi, though the λ_{max} of their absorption spectra were somewhat different from those of the Pg.

It is, therefore, inferred that the Pg is originated from the metabolites of *C. graniforme, A. tenuis* and probably other fungi and is contained not only in sclerotia but also in black mycelia (Hurst and Wagner, 1969). From the findings presented by Vol'nova and Mirchink, it may be reasonable to suppose that many species of fungi are sources of P type, but *C. graniforme* should be the first among them because of its broad distribution as a mycorrhiza-former.

4.4. Chromophore of Pg

Evidence indicating that the chromophore of the Pg is 4,9-dihydroxyperylene-3,10-quinone (DHPQ) is as follows (Sato and Kumada, 1967):

(1) P type HA was extracted from an Alpine meadow soil with 0.1 N NaOH. Pg was separated from the remaining brown HA by chromatography on columns of cellulose powder, and purified by gel filtration with Sephadex G-50. Also, a sclerotial Pg was obtained from sclerotia collected from the H horizon of an Alpine podzol by the same procedure.

The benzene extract of the zinc dust fusion products of soil Pg was separated by TLC on silica gel plates with *n*-hexane. A strong blue UV fluorescent band at R_f 0.5 was scraped off and a yellow hydrocarbon was recovered from the gel by elution with *n*-hexane. This hydrocarbon, purified by repeating the same TLC twice, has a UV spectrum characteristic of perylene (Fig. 4-6) and the same compound was also obtained after zinc dust fusion of sclerotial Pg.

(2) A purified fraction of Pg was obtained as a red pigment by extracting soil or sclerotial Pg with hot ethanol and concentrating the extract. The pigment is emerald green in aqueous alkali, and its λ_{max} was identical with that of Pg. As shown in Fig. 4-7, the absorption spectrum of the red pigment was very similar in shape to that of synthetic DHPQ except that there was a bathochromic shift. Like the synthesized quinone, alkaline solutions of Pg and red pigment change from green to pinkish red on reduction with sodium borohydride or sodium hydrosulphite, and this color change is easily reversed by aeration. These results suggest that Pg and the red pigment are derivatives of DHPQ, and that the red pigment is probably the major chromophoric component of Pg.

The occurrence of perylenequinones as fungal metabolites has been reported in several cases (*e.g.*, Weiss *et al.*, 1965; Yamazaki and Ogawa, 1972; Okuno, 1983), and all of the described compounds include DHPQ as part of their basic skeletal configuration. The red pigment dealt with here

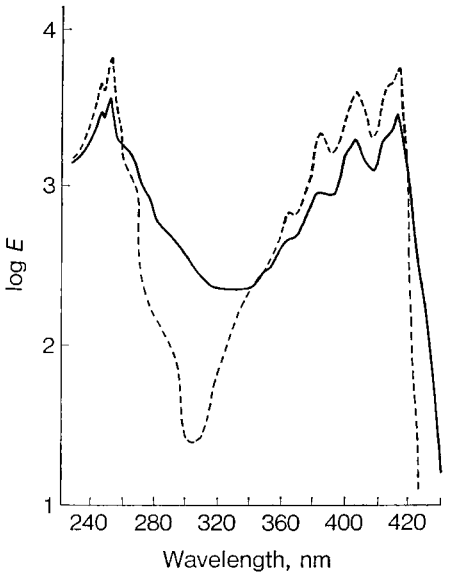


Fig. 4-6. Absorption spectra of the yellow hydrocarbon obtained from zinc dust fusion products of Pg (——) and perylene (- - -).

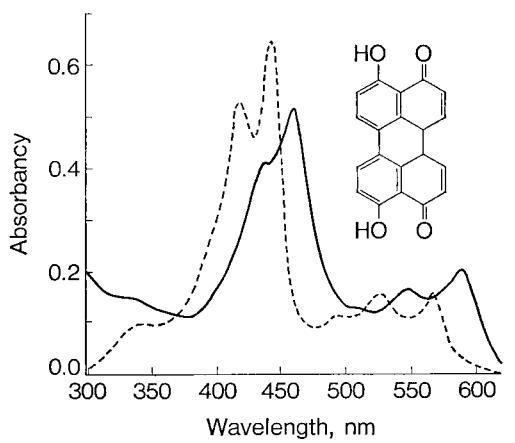


Fig. 4-7. Absorption spectra of Pg (——) and 4,9-dihydroxyperylene-3,10-quinone (- - -) in tetrachloroethane • ethanol (95:5).

almost certainly belongs to this group, but differs in its spectral properties from any fungal pigment so far reported.

Pg obtained from different soil types and sclerotia at different sites

showed the same λ_{max} in aqueous alkali, presumably indicating that the Pg is one and the same metabolite of a specific sclerotium-producing fungus or fungi belonging to the same genus. *C. graniforme* is the first candidate, as mentioned above. Attempts to separate Pg into components which produce clear-cut spots on TLC plates or to obtain "pure" Pg have been unsuccessful. This may be understandable since the Pg is a polymer having relatively lower molecular weight and containing a DHPQ nucleus.

4.5. Other Soil Quinone Pigments

In 1967, McGrath isolated a hydroxyanthraquinone pigment from the Bh horizon of Screen Podzol in Ireland, and showed its widespread distribution, not only in podzolized soil but also in Brown earth of the country (McGrath, 1967). His subsequent investigation on the chemical nature of the pigment resulted in a new type of dehydrodimer of chrysophanol called chrysotalunin (McGrath, 1970). He reported, furthermore, that chrysotalunin was occasionally present at over 120 ppm, and that chrysophanol and physcion were also identified in the soil (McGrath, 1972).

Subsequently, the author searched for hydroxyanthraquinone pigments in soils of Japan and confirmed the presence therein of chrysotalunin, chrysophanol, chrysazin and skyrin (Matsui and Kumada, 1974). The presence of oxyskyrin was presumed. Chemical structures of these pigments are shown in Fig. 4-8.

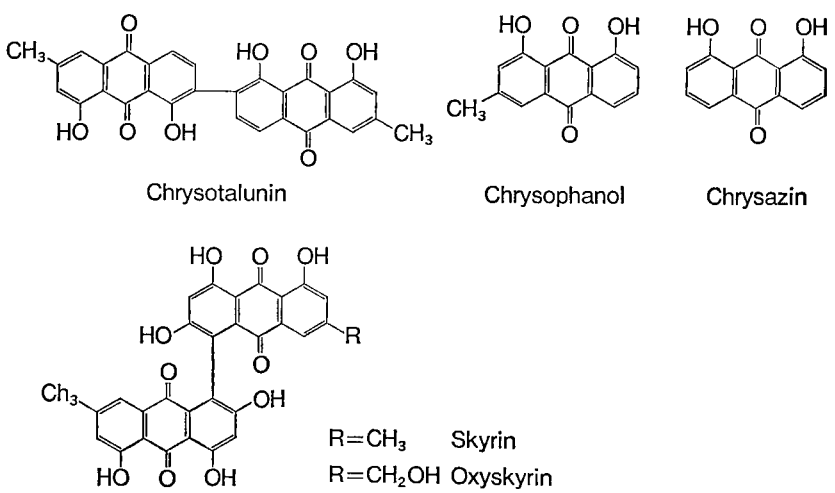


Fig. 4-8. Chemical structures of pigments investigated.

The amounts of hydroxyanthraquinones in eight soils were determined by colorimetry using chrysotalunin as a standard pigment. This pigment was kindly donated by Dr. D. McGrath. Total amounts of hydroxyanthraquinones were about 11 mg/kg in several soils, which is not greatly different from 15 mg/kg soil reported in the Bh horizon of Screen Podzol. The main pigment in the extracts from most soils was chrysotalunin, although the fact that this material was not detected in all soils might suggest that its distribution has some characteristic pattern. The presence of chrysotalunin and/or other hydroxyanthraquinones was thus confirmed in both Irish and Japanese soils. This also tends to confirm the common distribution throughout the world, as does the Pg fraction.

Many investigations have been carried out to characterize the chemical nature of soil HA. Several researchers found polynuclear hydrocarbons in the reductive distillation and fusion products of HAs with zinc dust (Sato and Kumada, 1967; Cheshire *et al.*, 1967; Hansen and Schnitzer, 1969; Kumada and Matsui, 1970); these findings suggested the presence of a hard core of polynuclear aromatics in HAs (Cheshire *et al.*, 1967). However, it is not clear whether the polynuclear aromatics were derived from an integral part of the hard core or from the components of low molecular weight, such as soil pigments of polynuclear structures, because the yields of polynuclear hydrocarbons reported to date have been extremely low. There is a possibility that the polynuclear hydrocarbons obtained from HA were derived from co-extracted impurities of low molecular weight. At the same time, there is another possibility that they were derived from polynuclear rings consisting of a turbostratic structure. Kumada *et al.* (1961) identified anthraquinone and 2-methyl naphthalene in the decarboxylation products of the water soluble acid fraction obtained by the alkaline permanganate oxidation of HAs. But the yields of these compounds were very low, and their sources can be explained by either of the two possibilities mentioned.

A number of publications have appeared on quinone pigments in soils. Butler *et al.* (1964) isolated a pigment from irregular olive green patches in Australian lateritic and podzolic soils. This pigment is a hexachloro-polynuclear quinone which has been assigned 1,3,6,8,11,13-hexachloro-4,10-dihydroxydinaphtho[2,1-b:1′,2′-*d*]furan-5,9,dione (Fig. 4-9, Cameron and Sidell, 1978; Cameron *et al.*, 1978). Lambert *et al.* (1971) extracted 69 mg of 2-methoxy-1,4-naphthoquinone from 8 kg of a high-montmorillonite tropical soil. In this connection, it is interesting that Mathur (1971), using cultures and an enzyme preparation of *Poria subacida*, obtained yields of 2-methyl-1,4-naphthoquinone up to 10% of a podzol FA preparation that had previously been studied by Hansen and Schnitzer (1967).

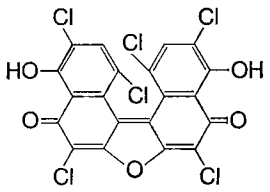


Fig. 4-9. Chemical structure of a polychloroquinone soil pigment. (Cameron and Sidell, 1978).

In the natural world, a large number of quinone pigments are formed as the metabolites of animals, plants, and microorganisms, and most of them reach the soil. As reviewed by Haider *et al.* (1975), various quinone pigments, especially polynuclear quinones such as hydroxyanthraquinones (Saiz-Jimenez *et al.*, 1975) and dihydroxyperylenequinones, are produced as fungal metabolites and transformed into dark polymers. These processes must be occurring in the soil. Therefore, it seems curious that the pigments actually detected in soils are very few with respect to kind and quantity. This suggests that the quinone pigments are rapidly decomposed by soil microorganisms, with only a small part of them being incorporated in the humus. Various degradation experiments conducted on HAs have rarely succeeded in obtaining polynuclear aromatics, and then only in low yields. This is a serious problem in humus chemistry, and will be discussed in the last chapter.

As for the sclerotia dealt with in Section 4.1, most are hollow and float in the water. Therefore, some may be washed out from the soils by rain or thawing water, floated down the river, carried to lakes or the sea, and deposited there. The author had the experience of gathering dozens of grams of sclerotia floating in thawing water. Thus, the Pg in sclerotia as well as in soils may serve as one of the biogenic precursors of perylene in lake and sea sediments.

In this connection, Orr and Grady (1967) stated that perylene is presumably formed by reduction of peryhydroxyperylenequinone pigments of biological origin. The reduction to perylene takes place in anaerobic sediment after burial. It was suggested by Aizenshtat (1973) that perylene precursors arise predominantly from land organisms and are carried into oceanic traps.

Chapter 5

Elementary Composition of Humic Acids and Fulvic Acids

A large number of published papers have included the elementary composition of HAs and FAs, though comprehensive studies on their elementary composition *per se* are few. Among these few, Kononova in 1975 wrote: "At the present time, comprehensive information is available on the elementary composition of the HA isolated from different soils. Some investigators draw attention to a similarity in the values of this criterion in HAs of different origin. Gillam (1940), for instance, found no essential differences between the elementary composition of HAs isolated from forest soils, meadow soil, and manured soil. Tischenko and Rȳdalevskaya (1936), however, were able to show regular changes in the elementary composition of HAs, reflecting the natural conditions under which humus was formed, in a series of soils ranging from podzolic soils to chernozems and further, to chestnut soils; in the HAs of this soil series it was found that the percentage of carbon increased and that of hydrogen and oxygen decreased, the decrease of hydrogen being greater than that of oxygen so that the O:H ratio became wider. . . . The data presented by Tischenko and Rȳdalevskaya were confirmed by other investigators."

Using a large number of analytical data on HAs and FAs, Orlov (1985) discussed in detail the variability of the elementary composition of HAs within the same soil type, the relationships between elementary composition and soil type, graphico-statistical analysis, and the degree of oxidizability of HAs. His interesting and valuable findings deserve careful examination.

Schnitzer (1977) conducted elementary analyses of HAs and FAs extracted from soil formed under widely differing geographic and pedologic conditions. He stated that FAs contain more oxygen and sulfur but less carbon, hydrogen, and nitrogen, and that the elementary composition of HAs

and FAs obtained from soils in the temperate zone did not differ from those in the tropical zone.

Kuwatsuka *et al.* (1981) statistically studied relationships between elementary compositions and types of HAs or the degree of humification, using 39 HAs prepared from various types of soils and wrote: "(i) Mean values of C%, H%, O%, H/C, N/C, and O/C of the different types of HAs (A, B, Rp(1), Rp(2), and P_0) were compared. The elementary composition of various types were proven by variance analysis to be significantly distinct. (ii) According to linear regression analyses, significant correlations (0.1 to 5% level) were found between C%-*RF*, H%-*RF*, H%-Δlog *K*, H/C-*RF*, H/C-Δlog *K*, N/C-*RF*, N%-*RF*, N%-Δlog *K*, N/C-Δlog *K*, and O%-*RF*, while no significant relationship was found with regard to C%-Δlog *K*, O%-Δlog *K*, O/C-*RF*, and O/C-Δlog *K*. (iii) Carbon and oxygen contents of HAs may be apt to reflect the different conditions of soils. (iv) The deeper the visible light absorption of HAs and so the higher the degrees of humification, the lower the hydrogen contents of HAs were. Though nitrogen content showed a trend similar to that of hydrogen content against *RF* values as a whole, the nitrogen content of less humified HAs (Rp type) varied from very low to very high values, suggesting the enrichment of nitrogen into HA molecules in the early stage of humification. (v) In the H/C *vs.* O/C diagram, HAs occupied an area reserved for the oxidation products of lignins. The area was fairly wide and "J"-shaped, that is, in the early to the middle stage of humification HAs were arranged in the direction of dehydrogenation or demethanation, and in the latter stage they were arranged in the direction of dehydration."

As described in Chapter 3, Kumada (1985a) pointed out that there were highly significant relationships between the carbon content and hydrogen content or C/H ratio, and between the elementary composition and parameters characterizing absorption spectra for the HAs and FAs prepared from 4 Canadian and 2 Japanese soils.

These findings are still fragmentary, and there is not yet a complete understanding of the elementary composition of HAs and FAs. Generally speaking, the elementary composition of organic substances affords fundamental information on their chemical configuration. So far as HAs and FAs are concerned, however, their elementary composition varies considerably with the kind of soil. Furthermore, the elementary composition of HAs obtained from the soil sample may vary remarkably with the method of preparation, as illustrated later. Diversity in the elementary composition of HAs and FAs seems to have obstructed their systematic understanding.

Is it possible for us to discover the laws which govern the diverse elementary composition of HAs and FAs? What pedological significances can

we recognize in their diversity? Can the elementary composition of HAs and FAs provide insight to their chemical configurations and genesis?

With these critical concerns in mind, the author has examined certain items of the elementary composition of HAs and FAs in more detail. (1) Deviation of analytical values. (a) Analytical values of the elementary composition of HAs and FAs obtained from the same soil samples using the same preparation method. (b) Analytical values of the elementary composition of HAs obtained from soil samples collected at the same sampling site by different workers at different times. (2) Elementary composition of HAs obtained from soil samples by successive extraction with NaOH, NaF and $Na_4P_2O_7$ or NaOH and $Na_4P_2O_7$. (3) Attempts to establish methods for characterization of the elementary composition of HAs and FAs.

In the analysis of HA and FA elementary composition, carbon, hydrogen and nitrogen, and sometimes sulfur and phosphor are determined and oxygen is calculated by difference: $100-(C\%+H\%+N\%+S\%+P\%)$. In the following discussion, however, sulfur and phosphor were excluded, oxygen was calculated by difference: $100-(C\%+H\%+N\%)$, and the content of four elements, C, H, N, and O, was expressed as atomic number percent. The reasons for this are: (i) Sulfur and phosphor have not been determined at the author's laboratory. (ii) When elementary composition is expressed as weight percent of each element, hydrogen content is overestimated and nitrogen content is underestimated. (iii) When atomic number percent is adopted, sulfur and phosphor contents are in most cases negligible in comparison with contents of the remaining elements.

5.1. Deviation of Analytical Value

5.1.1. Elementary composition of humic acids and fulvic acids obtained from the same soil samples using the same method of extraction by the same workers

Analytical data of HAs and FAs obtained by repeating extraction from each 20 g of two soil samples (A horizon of Black soil (Inogashira) and Brown forest soil (Dando), <2 mm, air-dried soil) according to the IHSS (International Humic Substances Society) method outlined in Fig. 5-1 are given in Fig. 5-2 (Ito, 1985). Here, FA refers to the XAD-8 adsorbed, *i.e.*, colored substances in what has been designated, in previous reports, as FA. The XAD-non adsorbed substances were discarded. By repeating the procedures of preparation, 6, 5, 5, and 8 samples were obtained for Inogashira HA and FA and Dando HA and FA, respectively. Ash contents were lower than 1%, except for 4% level for one sample and 3% level for 3 samples.

There were trends in HAs obtained from each soil sample that, with

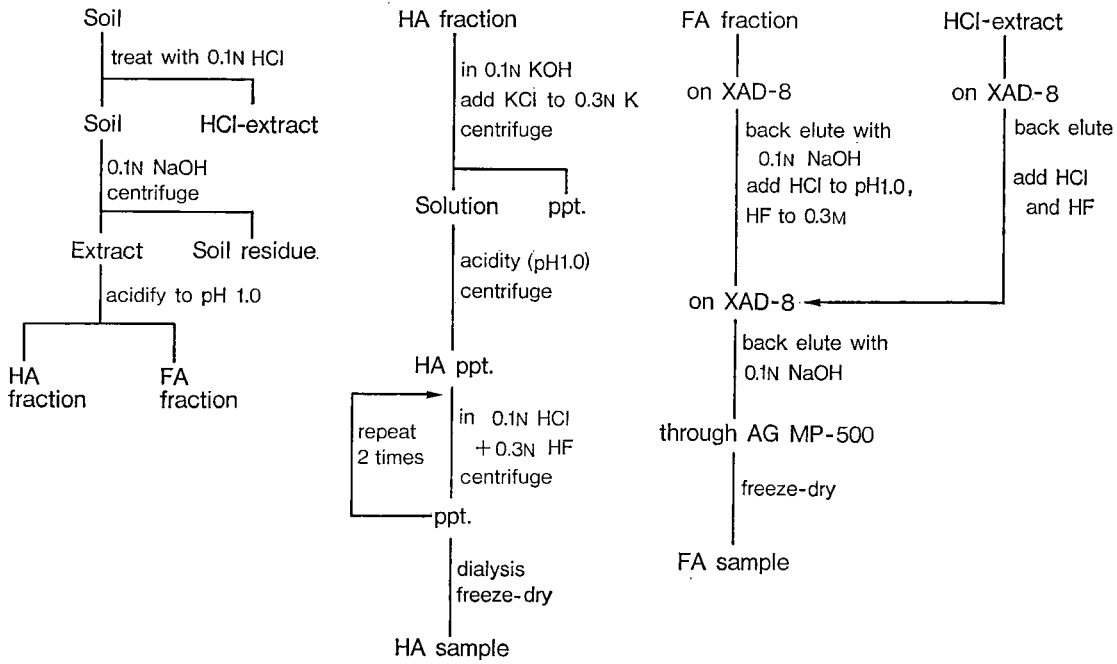


Fig. 5-1. Preparation of humic acid and fulvic acid samples (IHSS method).

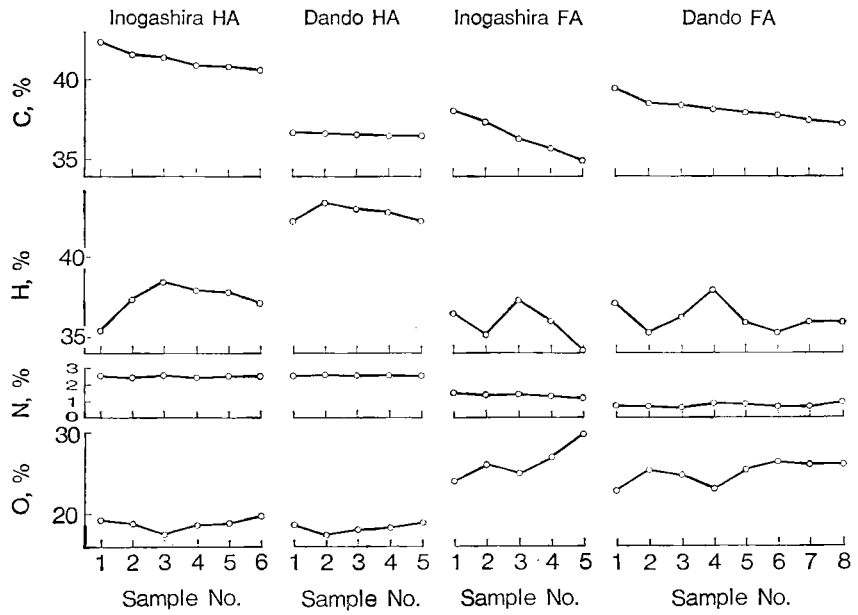


Fig. 5-2. Elementary composition of humic acids and fulvic acids obtained from the same soil samples by the same method (atomic number percent, ash and moisture-free basis, Ito, 1985).

the decrease of carbon content, the hydrogen content first increased and then decreased; the reverse was true in the case of oxygen. These trends were not observed in FAs. Fluctuation coefficients of the carbon and hydrogen contents were low, ranging from 0.2 to 3.4% for both HAs and FAs. Fluctuation coefficients of the nitrogen and oxygen contents were 0.6 to 2.9% for HAs and 5.4 to 12% for FAs.

The deviation of values obtained by repeated analyses for the same HA and FA samples is usually very small. The deviations of the above values, however, were large, suggesting that all the procedures included in the method of preparation resulted in a large deviation.

Dubach and Mehta (1963) stated that perhaps no two molecules of humic substances are exactly alike. Similarly, it may be said that we can never twice obtain exactly alike HA and FA even from the same soil sample.

5.1.2. Comparison of the elementary composition of humic acids obtained from the same soil sampling site

Table 5-1 shows the elementary composition of HAs used by Kuwatsuka *et al.* (1978), that of samples #5 and #6 from Table 3-1, the average elemen-

tary composition of HAs obtained from Inogashira and Dando soils by the IHSS method (Fig. 5-2), and that of HAs obtained from Inogashira and Dando soils by the Nagoya method (Ito, 1985). Included are several HAs which were obtained from different soil samples collected at the same site by different researchers at different times: 5 of Inogashira soil, 4 of Tsubame soil, and 2 each of Fujimiya and Dando soil.

Practical procedures used for the preparation of HA samples differed somewhat, except that 0.1 N NaOH was always used as extractant.

In Inogashira HAs, carbon and hydrogen contents ranged from 48.2 to 41.2% and 29.6 to 37.4%, respectively. These ranges were comparable with those of the A type HAs dealt with later. The fluctuation coefficients of carbon, hydrogen, nitrogen, and oxygen contents were 6.3, 9.0, 1.8, and 2.6 %, respectively, and distinctly higher than those of the HAs obtained from the same soil samples which are shown in Fig. 5-2. Regular relations were found among the elementary composition of these HAs that the higher the carbon content of an HA, the lower was its hydrogen and nitrogen contents and the higher its oxygen content. The same relations were also observed for Fujimiya, Tsubame and Dando HAs, the only exception being the nitrogen content of Fujimiya HA.

Sample Nos. 41 and 42 and Nos. 44 and 45 were obtained from the same soil samples, Inogashira and Dando by the IHSS and Nagoya methods, respectively, by the same workers. The data obtained for one sample coincided fairly well, despite differences in the methods of preparation. Accordingly, it is inferred that the widely disparate data for HAs obtained from different soil samples at the same site by different workers is due mainly to differences in the samples and workers, even though differences in practical procedures have various effects. Even if this inference is correct, it is impossible to explain the inversely proportional relations between carbon and oxygen contents and hydrogen and nitrogen contents for HAs from the same sampling site.

Orlov (1985) stated: "The data of many researchers point to a wide range of variation in the elementary composition of humic and fulvic acids isolated from soils of the same type and even from the same region. In some instances the fluctuations in the carbon content are larger than those typical of humic acids from different types of soil, . . . The variability of the elemental composition of humus acids is due to three groups of factors: 1) variability of soils and humus substances in time and space; 2) different techniques used to isolated humus substances from the soil; and 3) errors in sample preparation and in analytical procedure."

The three groups of factors pointed out by Orlov are reasonable and correct, but another factor(s) is necessary to explain the above proportional

TABLE 5-1
Elementary composition of various types of humic acid.

No.	Sample[a]	Soil[b] group	Origin[c]	HA type	Symbol	$\Delta \log K$	RF	Atomic percent				Atomic ratio				CQ
								C	H	N	O	H/C	N/C	O/C	O/H	
2	Tenmondai	Bl	1	A	●	0.527	144	47.5	29.6	2.1	20.8	0.62	0.044	0.44	0.70	1.11
6	Inogashira	Bl	2	A	●	0.485	115	48.2	29.6	1.5	20.0	0.61	0.031	0.43	0.70	1.09
4	//		1	A	●	0.496	138	44.8	33.5	2.1	19.5	0.75	0.047	0.44	0.58	1.07
40	// (#5)		5	A	●	0.538	—	43.6	35.1	2.4	18.9	0.81	0.055	0.43	0.54	1.06
41	//		6	A	●	0.506	111	41.9	36.7	2.6	18.8	0.88	0.062	0.45	0.51	1.06
42	//		7	A	●	—	—	41.2	37.4	2.5	18.9	0.91	0.061	0.46	0.51	1.05
8	Shitara black	Bl	1	A	●	0.547	114	45.9	31.6	2.3	20.2	0.69	0.050	0.44	0.64	1.09
10	Fujimiya	Bl	3	A	●	0.511	97	41.2	36.2	2.3	20.3	0.88	0.056	0.49	0.56	1.07
15	//		2	A→B	◑	0.541	82	39.6	40.4	1.8	18.1	1.02	0.045	0.46	0.45	1.01
13	Komagahara	Bl	3	A	●	0.542	89	41.7	34.4	2.0	21.9	0.83	0.048	0.53	0.64	1.10
26	Onobaru	Bl	3	P_0	◐	0.678	39	37.6	41.1	2.8	18.5	1.09	0.067	0.49	0.45	1.03
3	Nagara P	R	1	A	●	0.555	140	47.9	25.4	1.7	25.0	0.53	0.035	0.52	0.98	1.18
9	// F			A	●	0.620	102	44.0	32.4	2.7	20.9	0.74	0.061	0.48	0.65	1.11
14	// OH			A	●	0.667	89	42.2	34.9	3.0	19.9	0.83	0.071	0.47	0.57	1.09
5	Jaana P	R	2	A	●	0.478	132	45.9	33.5	1.5	19.1	0.73	0.033	0.42	0.57	1.05
17	// F			B	◑	0.619	65	40.3	38.8	2.9	18.0	0.96	0.072	0.45	0.46	1.04
31	// OH			Rp(1)	⊕	0.714	30	34.2	45.4	3.8	16.6	1.33	0.111	0.49	0.37	0.99
7	Kinshozan P	R	1	A	●	0.543	115	43.4	34.3	1.7	20.6	0.79	0.039	0.47	0.60	1.07
19	// F			B	◑	0.731	60	40.8	38.3	2.4	18.5	0.94	0.059	0.45	0.48	1.04
32	// OH			Rp(1)	⊕	0.851	29	36.9	43.0	1.9	18.1	1.17	0.051	0.49	0.42	0.99
12	Kinshozan P	R	3	A	●	0.565	92	43.0	32.5	1.7	22.7	0.76	0.040	0.53	0.70	1.12
39	// OH			Rp(1)	⊕	0.897	16	33.9	46.7	3.3	16.1	1.38	0.097	0.48	0.35	0.97

23	Ishimakisan P	R	2	P_0	◐	0.656	43	37.5	45.5	1.8	15.2	1.21	0.048	0.41	0.33	0.94
28	〃 F			Rp(1)	⊕	0.729	37	39.0	41.9	2.1	17.0	1.07	0.054	0.44	0.41	0.99
34	〃 OH			Rp(1)	⊕	0.804	22	36.3	45.2	2.5	16.0	1.25	0.069	0.44	0.35	0.96
16	Shitara brown	B	1	B	◑	0.605	76	38.3	41.5	2.6	17.7	1.08	0.068	0.46	0.43	1.01
18	Kuragari	B	1	B	◑	0.634	61	40.0	40.4	2.5	17.1	1.01	0.063	0.43	0.42	1.01
24	Kuragari	B	2	P_0	◐	0.678	43	34.6	50.0	1.9	13.5	1.45	0.055	0.39	0.27	0.89
21	Higashiyama (A)	B	1	$B_{\pm}$	◑	0.664	47	39.2	40.5	1.9	18.4	1.03	0.048	0.47	0.45	1.01
20	Anjo black	P/Bl	1	B	◑	0.642	57	39.1	40.5	2.5	17.9	1.04	0.064	0.46	0.44	1.02
29	Sanage	P	1	Rp(1)	⊕	0.771	32	36.9	43.8	3.3	16.0	1.19	0.089	0.43	0.37	0.99
27	Sanage	P	2	P_0	◐	0.657	38	37.3	45.1	2.5	15.1	1.21	0.067	0.41	0.34	0.95
35	Gifu	P	1	Rp(1)	⊕	0.853	22	35.7	44.4	3.3	16.6	1.24	0.092	0.47	0.37	0.99
36	Anjo	P	1	Rp(1)	⊕	0.835	21	36.7	43.0	3.1	17.2	1.17	0.084	0.47	0.37	1.00
30	Higashiyama (FH)	A_0	1	Rp(2)	○	0.766	31	38.2	42.0	2.0	17.8	1.10	0.052	0.47	0.42	1.00
33	Higashiyama (L)	A_0	1	Rp(2)	○	0.809	23	38.4	44.2	1.8	15.6	1.15	0.047	0.41	0.35	0.95
37	Yamamomo	RW	2	Rp(2)	○	0.968	21	40.2	40.9	0.6	18.3	1.02	0.0016	0.46	0.45	0.98
38	Kisokoma (F)	A_0	1	Rp(2)	○	0.806	20	37.5	45.4	3.3	13.8	1.21	0.088	0.37	0.30	0.95
11	Tsubame	BH	4	$A_{++} \rightarrow P_{++}$	△	0.469	94	41.7	36.1	1.8	20.4	0.87	0.043	0.49	0.57	1.06
43	Tsubame (#6)		5	P_{++}	△	0.382	—	40.1	39.5	1.9	18.5	0.99	0.047	0.46	0.47	1.02
22	Tsubame		1	P_+	△	0.513	69	38.2	42.4	2.1	17.2	1.11	0.055	0.45	0.41	0.99
25	Shishi	AGL	1	P_+	△	0.506	42	35.6	48.0	2.1	14.2	1.35	0.059	0.40	0.30	0.91
44	Dando	B	7	P_+	△	—	—	36.5	42.7	2.6	18.2	1.17	0.071	0.50	0.43	1.01
45	Dando		6	P_+	△	0.594	46	36.0	43.9	2.9	17.2	1.22	0.080	0.48	0.39	0.99
1	Tsubame	BH	4	Pg		0.272	202	48.8	25.4	0.94	24.9	0.50	0.019	0.51	0.98	1.16

[a] P, F, and OH denote the extractants for successive extraction, *i.e.*, P 0.1 M $Na_4P_2O_7$, F 0.1 M NaF, OH 0.1 M NaOH. (L), (F), (FH), (A) denote the horizons of forest soil.

[b] Bl: Black soil; R: Rendzina-like soil; B: Brown forest soil; P: paddy soil on alluvial land; P/Bl: paddy soil on Black soil; A_0: A_0 horizon; RW: rotted wood; RH: buried humic layer; AGL: Alpine grassland soil.

[c] (1) HAs prepared by Shiroya and Kumada (1973), (2) HAs by Kumada and Kawamura (1968a), (3) HAs by Kuwatsuka *et al.* (1978), (4) HAs by Sato (1976a), (5) HAs by Kumada (1985a), (6) HAs by Ito (1985, Nagoya method), (7) HAs by Ito (1985, IHSS method).

relations. The HA of a soil should be considered an assembly of fractions whose elementary composition changes continuously in such a way that the higher the carbon and oxygen contents, the lower the hydrogen and nitrogen contents; the HA actually obtained is a part of the assembly, and this part varies more or less with the procedure adopted. In any event, the regular changes in elementary composition of HA should be kept in mind.

5.2. Elementary Composition of Humic Acids Obtained by Successive Extraction

Table 5-1 includes HAs which were obtained by successive extraction using NaOH, NaF and $Na_4P_2O_7$ or NaOH and $Na_4P_2O_7$ from 3 Rendzina-like soils, *i.e.*, Nagara, Kinshozan, and Ishimaki. Except for Ishimaki HAs, the elementary composition of the HAs extracted successively showed tendencies that the carbon and oxygen contents increased and the hydrogen and nitrogen contents decreased with the order of extraction, while at the same time the *RF* values increased and $\Delta \log K$ values decreased. For the HAs of Ishimaki soil, no regular change in elementary composition was observed, probably due to their immaturity, as shown by the low carbon content and small *RF* value of the $Na_4O_2P_7$-extracted HA.

These findings support the idea just described above, which may be further developed: An HA of a soil horizon is an assembly of fractions whose elementary composition, optical properties and combination with polyvalent bases change continuously, and the following relations exist among these factors.

Degree of humification		Low ⟶ High
Optical properties	*RF*	Increase
	$\Delta \log K$	Decrease
Elementary composition	Carbon and oxygen	Increase
	Hydrogen and nitrogen	Decrease
Combinative ability with polyvalent bases		Increase

The successive extraction with NaOH, NaF, and $Na_4P_2O_7$ extracts the HA assembly from the fraction with lower degree of humification to the fraction with higher degree of humification one after another. The distribution of the properties in HA fractions probably varies with respective HA assemblies. This idea was supported by a study of the fractionation of HAs using a fractional precipitation technique with alcohol (Section 9.4; Kumada and Kawamura, 1968). That is, HAs of various types were separated into eight fractions by the alcohol precipitation technique and their *RF* and $\Delta \log K$ values were determined. The distribution pattern of the fractionated HAs and their *RF* and $\Delta \log K$ values varied with the degree of humification and type

of original HAs. It was tentatively concluded that *RF* value of fractionated HAs decreases and $\Delta \log K$ increases with decreasing particle weight of fractionated HAs in a given HA.

The above working hypothesis on the form of existence of HA is applicable to not only calcareous soils such as Chernozems, Grumusols, and Rendzinas, but also to the acid, base-deficient soils which prevail in Japan. The HAs of Black soils are exceptional; they form an Al-humus complex and a large part of it is extracted with NaOH.

The elementary composition, optical properties, and combinative ability with polyvalent bases of HA assembly of a soil may not always or not strictly correspond; the matter should be investigated further.

5.3. Methodology for the Comparison of Elementary Composition

Elementary composition varies remarkably with each HA, with the content of each element ranging widely. To discover the laws which govern this diversity of elementary composition, a methodology for the study should first be considered.

The author tried two approaches: firstly, to find suitable parameters and diagrams for characterizing the elementary composition and, secondly, to evaluate the parameters and diagrams in connection with the types of HA.

(1) Parameters and diagrams

The carbon, hydrogen, nitrogen, and oxygen contents themselves as well as the H/C, N/C, and O/C ratios can be regarded as parameters. The H/C-O/C diagram has been used in the field of coal science, and also by Orlov (1985) and Kuwatsuka *et al.* (1978).

In addition, the following new parameters and diagrams were tested.

a. O/H and CQ ratios: As illustrated later, the O/H ratio is effective as an index of the oxidation degree of HA. The CQ ratio is defined as $4C/(4C+H-3N-2O)$, that is, the Combustion Quotient proposed by Tamiya (1932), and expresses the theoretical respiration ratio. This ratio is calculated on the basis of the contents of all four elements.

b. H/C-O/H diagram: This diagram seems more useful than the H/C-O/C diagram, as shown later.

c. C′-H′-O′ and H″-N″-O″ diagrams: C′, H′, and O′ contents calculated by putting the sum of C, H, and O contents at 100% are plotted on a triangular diagram. By neglecting N content which usually does not exceed 4%, each HA is characterized by its position on the figure. Similarly, H″, N″, and O″ contents calculated by putting the sum of H, N, and O contents at 100% are plotted on a triangular diagram. This figure is intended to characterize HA by the relative proportion of H, N, and O and also by enlarging

the N content. These attempts are intended to simplify comparison by graphic representation.

(2) Relationships between elementary composition and other properties

To deepen our knowledge of the elementary composition of HAs, it is useful to compare them in relation to other soil properties. For instance, Kononova (1975) studied the elementary composition of HAs in relation to soil zonality, Schnitzer (1977) compared HAs of the temperate zone with those of the tropical zone, and Orlov (1985) discussed the relationships between the elementary composition of HAs and soil types.

Following Kuwatsuka *et al.* (1978), relationships between elementary composition and HA type are discussed below.

5.4. Elementary Composition of Humic Acid and Fulvic Acid Samples

5.4.1. Humic acid samples

HAs used in the following considerations are listed in Tables 5-1 and 2. As mentioned, Table 5-1 includes HAs which were obtained from soil samples at the same sampling site by different workers; they are dealt with as different samples. Sample Nos. 10 and 15 were originated from the soils at Miyahara, and designated as A type; but the elementary composition of No. 15 was in the range of B type, so it is dealt with as B type. Sample No. 11 was designated as A_{++} type but regarded as P_{++} type, because of its strong Pg absorption.

Recently, Matsui *et al.* (1986) investigated the diagenesis of HAs of Black soils. Table 5-2 is the data on the elementary composition as well as optical properties and X-ray diffraction of the HAs obtained from the present and past (buried) A horizons. Although the analytical results will be discussed in detail in Chapter 11, the data on elementary composition are utilized here.

The assignment of type was impossible for HAs listed in Table 5-2, because their *RF* values had not been determined. Based on the experimental results on optical properties and X-ray diffraction, however, it is certain that samples Hy 1, Hy 2, and Ka 1 belong to B or Po type, and the rest to A type. Among the HAs belonging to A type, the ones obtained from buried humic horizons were designated as bA type for convenience.

5.4.2. Attempts to characterize the elementary composition of humic acids by various means

Ranges of the parameters of humic acids of respective types

The contents of carbon, hydrogen, nitrogen, and oxygen, and the ratios of H/C, N/C, O/C, O/H, and CQ of the HAs listed in Tables 5-1 and 2, except

TABLE 5-2

Parameters of the elementary composition, optical properties, and X-ray diffraction of humic acids obtained from buried humic horizons of Black soils.

Sample	Horizon	Age	Symbol	Atomic percent				Atomic ratio				CQ	$E_6$1%	E_4/E_6	d	n
				C	H	N	O	H/C	N/C	O/C	O/H					
Hy 1	A	1739 A.D.	○	36.61	41.22	2.43	19.74	1.13	0.066	0.54	0.48	1.04	21.9	5.14	4.5	—
Hy 2	II AC	1667–1739 A.D.	○	36.91	42.11	2.51	18.47	1.14	0.068	0.50	0.44	1.02	30.3	3.81	4.5	—
Ka 1	A_{11}		○	37.20	42.96	2.75	17.09	1.16	0.074	0.46	0.40	1.00	44.5	3.52	4.4	—
Ka 2	A_{12}		●	39.75	37.52	2.40	20.33	0.94	0.060	0.51	0.54	1.07	75.0	3.23	3.7	2.32
Hy 3	II A	1640–1667 A.D.	■	40.67	36.31	2.35	20.67	0.89	0.058	0.51	0.57	1.08	44.7	4.11	3.6	2.33
Ka 3	II A	1,000 y.B.P.	■	44.16	33.62	1.90	20.32	0.76	0.043	0.46	0.64	1.08	93.3	3.27	3.6	2.60
Yu 2	II A	800–864 A.D.	■	47.85	27.57	1.70	22.88	0.58	0.036	0.48	0.83	1.14	90.7	3.26	3.5	2.59
Si 1	A_{11}		●	43.72	27.89	1.65	26.74	0.64	0.038	0.61	0.96	1.21	93.1	3.27	3.5	2.67
Si 2	A_{12}		●	46.03	24.03	1.43	28.50	0.52	0.031	0.62	1.19	1.25	120	3.16	3.5	2.74
Ka 4	III A	3,000 y.B.P.	■	47.50	26.91	1.29	24.30	0.57	0.027	0.51	0.90	1.16	109	3.21	3.5	2.55
Si 3	II A_{11}	3,000 y.B.P.	■	46.74	23.99	1.27	28.00	0.51	0.027	0.60	1.17	1.24	105	3.30	3.5	2.76
Yu 3	IV A	3,500	■	51.67	22.88	1.20	24.25	0.44	0.023	0.47	1.06	1.17	113	3.19	3.5	2.68
Ka 5	IV A	4,000	■	47.35	28.98	1.39	22.28	0.61	0.029	0.47	0.77	1.12	66.7	3.10	3.5	2.57
Si 4	II A_{12}	4,500*	■	45.68	24.83	1.26	28.22	0.54	0.028	0.62	1.14	1.24	106	3.31	3.5	2.72
Si 5	III/IV A	6,040*	■	47.21	23.18	1.21	28.40	0.49	0.026	0.60	1.23	1.25	110	3.44	3.5	2.72
Yu 4	VI A	7,000*	■	48.14	23.90	1.05	26.91	0.50	0.022	0.56	1.13	1.21	121	3.05	3.5	2.67
Hy 4	IV A	8,940*	■	47.38	28.28	2.10	22.24	0.60	0.044	0.47	0.79	1.14	83.9	3.64	3.5	2.69
Yu 5	VI A	10,000	■	51.36	22.11	0.91	25.62	0.43	0.018	0.50	1.16	1.18	128	3.10	3.5	2.80
Si 6	IV A	10,000	■	46.75	23.99	1.20	28.06	0.51	0.026	0.60	1.17	1.24	100	3.54	3.5	2.76
Si 7	VI A	18,000*	■	45.37	24.86	1.12	28.65	0.55	0.025	0.63	1.15	1.25	95.8	3.64	3.5	2.66
Si 8	VII A	22,000	■	47.13	23.18	1.14	28.55	0.49	0.024	0.61	1.23	1.25	98.4	3.49	3.5	2.72
Si 9	VIII A	26,000	■	47.07	23.19	0.99	28.75	0.49	0.021	0.61	1.24	1.25	98.6	3.62	3.5	2.72

* Determined by the ^{14}C method.

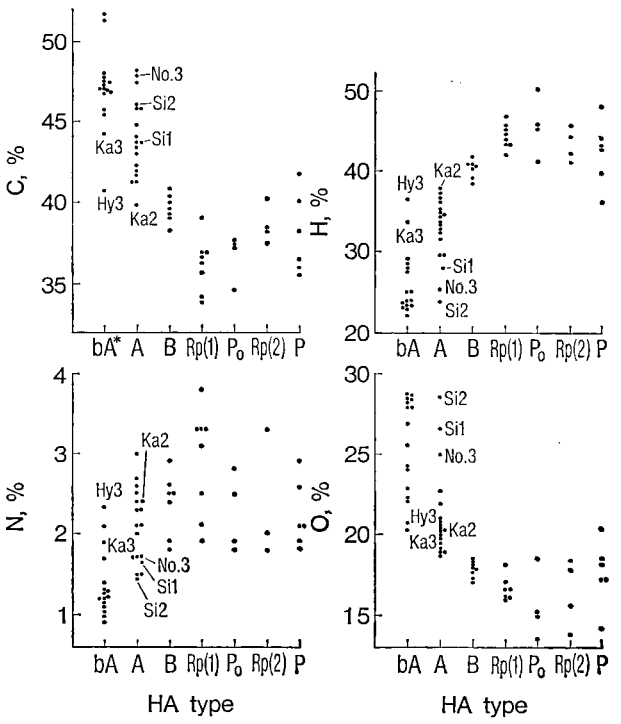
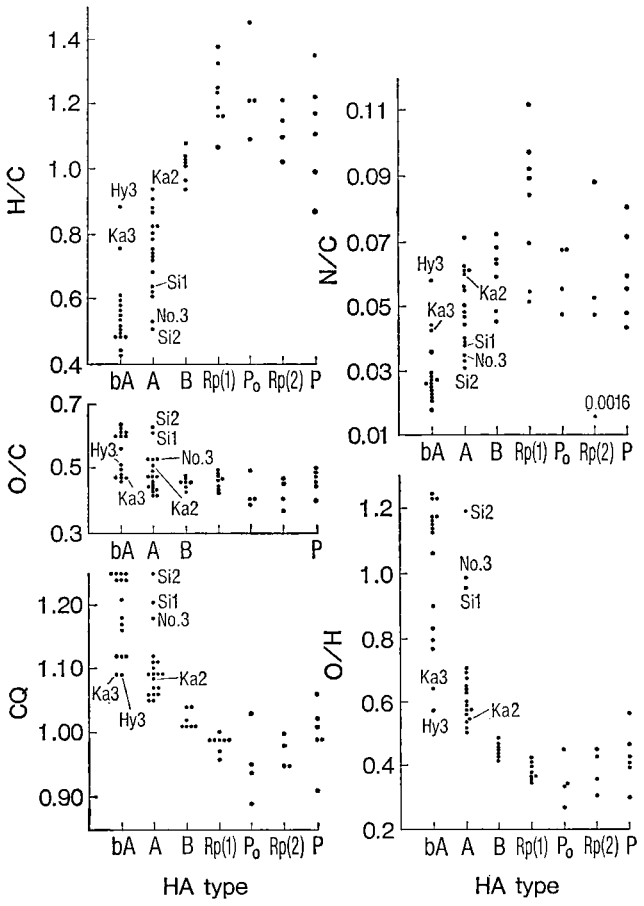


Fig. 5-3. Elementary composition
* bA type means A type HA obtained

for Hy 1, Hy 2, and Ka 1, are plotted in Fig. 5-3. In the figure, samples No. 3, Ka 2, Si 1, and Si 2 obtained from present A horizons and Hy 3 and Ka 3 obtained from younger buried humic horizons are discriminated from the rest by indication of the sample name.

As seen, there are tendencies for HAs called bA type to have higher C, O, O/C, O/H, and CQ values and lower, H, N, H/C, and N/C values than those of A type HAs. When samples Ka 3 and Hy 3 are moved to A type, and No. 3, Si 1 and Si 2 to bA type, the differences in elementary composition between the A and bA type HAs become more distinct, and they are almost completely discriminated from each other in all parameter ranges.



of various types of humic acid.
from buried humic horizon of Black soils.

These findings may be interpreted as follows: (i) Generally speaking, A type HAs characterizing matured Black soils transform to bA type with the lapse of time after burial as will be discussed in Chapter 11. (ii) HAs of younger ages, *e.g.*, Hy 3 (1640–1667 A.D.) and Ka 3 (*ca.* 1,000 y.B.P.), still belonged to A type, while samples Si 1 and Si 2 obtained respectively from A_{11} and A_{12} horizons belonged to bA type. These facts suggest that the elementary composition of the HAs of buried horizons aged 1,000 y.B.P. or younger are in the range of that of present A horizons and, further, that A type will be transformed into bA type with respect to elementary composition when sufficient time (presumably 1,000 years) has passed, even if burial by volcanic fallout does not occur.

Sample No. 3 is the HA extracted with $Na_4P_2O_7$ after successive extraction with NaOH and NaF from a Rendzina-like soil, and showed the highest degree of humification among the HAs in Table 5-1. This example may indicate that A type HA originating from Rendzina-like soil also transforms into bA type after a certain time has passed.

Although the elementary composition of sample Ka 2 (A_{12} horizon) was in the A type range, the carbon content was lowest and the hydrogen content and H/C value highest, suggesting its immature A type character.

Carbon contents of B type HAs were completely distinguished from those of the A type; the same was true of Rp(1) and P_0 type HAs, with one exception of Rp(1) type. The carbon contents of the Rp(1) and P_0 type HAs appeared to overlap each other, and their ranges were different from the Rp(2) HAs. As for the hydrogen and oxygen contents, the ranges of these elements were generally distinguishable among the bA, A, B, and Rp(1) type HAs, and were all fairly narrow. Ranges of the hydrogen and oxygen contents of the P_0 and Rp(2) type HAs were wide and overlapped each other. Ranges of the nitrogen content of the respective types were also wide and overlapped, although that of the bA type HAs tended to be low.

In P type HAs, the contents of all four elements showed wide ranges. As shown in the bottom row of Table 5-1 and Table 4-1, the elementary composition of the Pg fraction was characterized by high carbon and oxygen contents and low hydrogen and nitrogen contents. Thus, the wide ranges of these elements of P type HAs seem to reflect the relative amount of the Pg fraction.

Situations similar to the hydrogen and nitrogen contents were respectively observed in H/C and N/C ratios among various types of HA. In the O/C ratios, however, those of bA type HAs tended to be higher than those of other types, but no differentiation was possible among the other types. On the other hand, the O/H ratio lowered in the order of bA type, A type, B type, and Rp(1) type, and those of the other types were comparable to the Rp(1) type.

The fact that no significant difference in O/C ratios was observed among the various types can be explained by the tendency for an HA with high carbon content to have high oxygen content. Remarkable differences observed in the O/H ratios among various types indicated that the ratio serves as parameter for characterizing the HA elementary composition.

With respect to the CQ values, the bA, A, B, and Rp(1) types were almost completely distinguishable from each other; ranges of these values for other types were wide and overlapped equally. The similarity of the distribution patterns of the O/H and CQ ratios among various types is interesting, because the significance of these values is entirely different. As a highly

significant correlation coefficient was obtained between O/H and CQ values (Section 5.5), CQ can be replaced by O/H.

The results described above indicate that the ranges of the parameters of the elementary composition of HAs vary considerably with the type of HA. That is, bA and A types were completely distinguishable from other types with respect to carbon, hydrogen, and oxygen contents, H/C, O/H, and CQ ratios. Between the bA and A types, the ranges of carbon contents and H/C ratios overlapped considerably, but those of hydrogen and oxygen contents, and O/H and CQ ratios were almost completely separated. The B and Rp(1) types were almost completely distinguishable from each other with respect to carbon, hydrogen, and oxygen contents and H/C, O/H, and CQ ratios. Differentiation between the Rp(1) type and the remaining Rp(2), P_0, and P types was impossible. The nitrogen content of only bA types tended to be lower than that of other types.

H/C-O/C and H/C-O/H diagrams

Based on the data in Tables 5-1 and 2, Fig. 5-4 (H/C-O/C diagram), and Fig. 5-5 (H/C-O/H diagram) were drawn. The distribution areas of the bA, A, B, Rp(1) type HAs and the one comprising all HAs belonging to B, Rp(1), P_0, Rp(2) types were shown by linking the outermost HA of the same type(s); these are here referred to respectively as bA area, A area, B type area, Rp(1) type area and B area. To establish the boundaries of the respective areas, data on the elementary composition of the HAs obtained from Black soils having buried humic horizons reported by Ohtsuka (1974b)

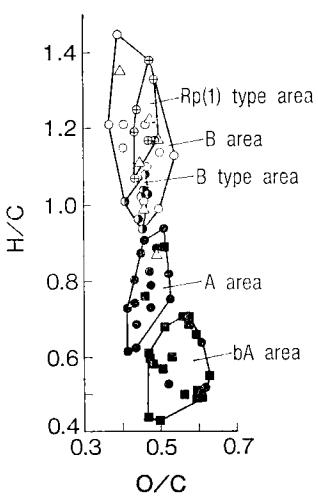


Fig. 5-4. H/C-O/C diagram of various types of humic acid. ■: bA type; ●: A type; ◑: B type; ⊕: Rp(1) type; ○: Rp(2)•P_0 types; △: P type.

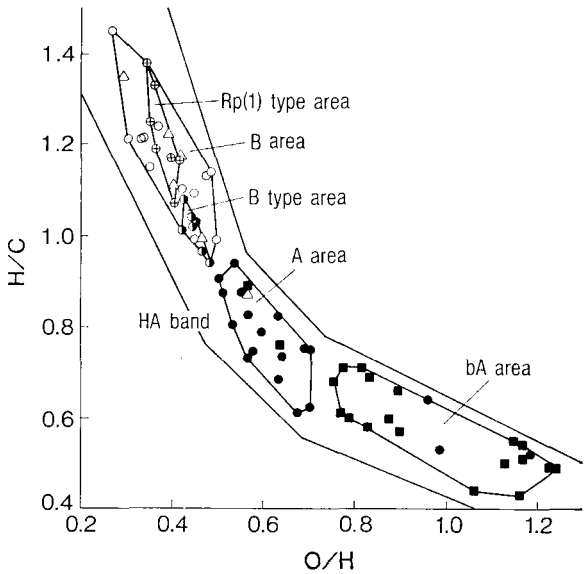


Fig. 5-5. H/C-O/C diagram of various types of humic acid. Notations are the same as in Fig. 5-4.

and Yoshida and Kumada (1984) were also utilized. Samples Hy 1, Hy 2, and Ka 1 were in the B area, Hy 3 and Ka 3 in the A area and Si 1, Si 2, and No. 3 in the bA area. Sample No. 11 (P_{++} type) was located in the A area, and the remaining P type HAs in the B area.

Both figures clearly show that the HAs belonging to the bA, A, and the other types form definite respective areas which do not overlap. The B and Rp(1) type HAs each constitute a narrow area included in the B area. As the sample number of HAs belonging to the B, Rp(1), P_0, Rp(2), and P types are all very limited, it may be safe at present to lump them all together as the B area.

It is evident that the H/C-O/H diagram can discriminate among the bA, A, and B areas more clearly than the H/C-O/C diagram. Transition from the B area to the A area and further to the bA area is considered to reflect the progress of humification of HAs and their subsequent diagenesis under terrestrial conditions, respectively. Therefore, the long and slender band which encloses all three areas may be called the HA band. This band is characterized by the descent of the H/C and ratio the ascent of the O/H ratio, and is entirely different from the so-called coal band. In other words, humification and coalification should be distinguished from each other. The humification of HAs and their subsequent diagenesis are characterized

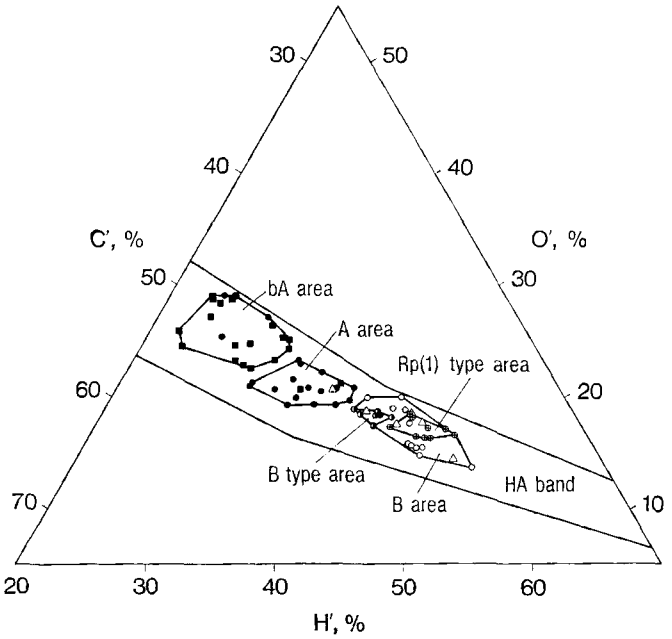


Fig. 5-6. C'-H'-O' diagram of various types of humic acid. Notations are the same as in Fig. 5-4.

by the processes which take place in the soil environments where oxygen prevails.

C'-H'-O' and H''-N''-O'' diagrams

The C'-H'-O' diagram drawn by the same procedures as Figs. 5-4 and 5-5 is shown in Fig. 5-6. Here too, the same bA, A, and B areas composed of the same HA members as those in Figs. 5-4 and 5-5 were obtained, and the B and Rp(1) type areas were included in the B area. Thus, the HA band can be similarly drawn. In the HA band, the transition from the B area to the A area and from the A area to the bA area indicates the progress of humification and diagenesis of HAs, respectively. As a whole, the direction of the transition seems to point to the enrichment of carbon and oxygen and the reduction of hydrogen in HAs.

The fact that the distribution patterns of HAs in Figs. 5-4 and 5-5 are the same as those in Fig. 5-6 is understandable because these figures are in principle the same, and the H/C-O/H and H/C-O/C diagrams can be drawn on the C'-H'-O' diagram.

In the H''-N''-O'' diagram (Fig. 5-7) too, the bA, A, and B areas are

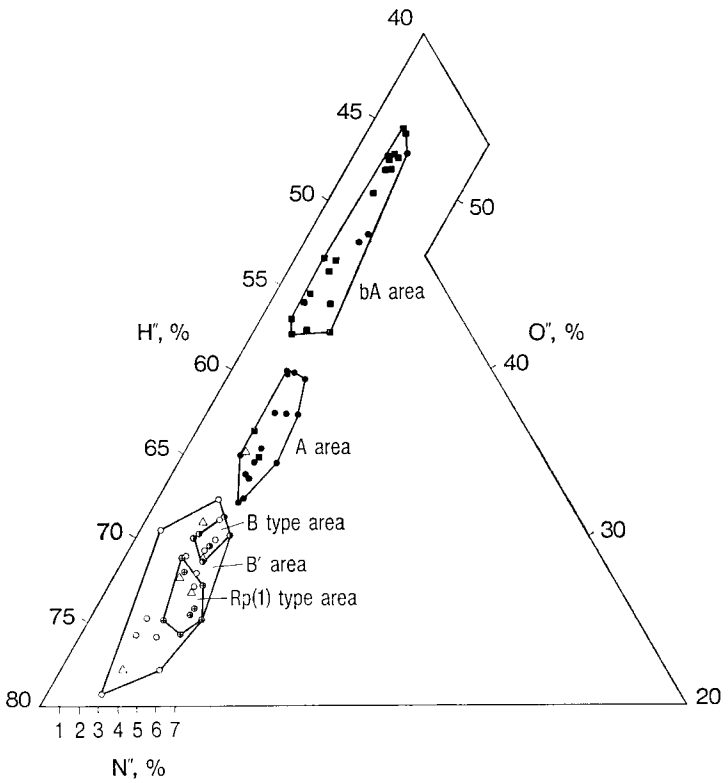


Fig. 5-7. H″-N″-O″ diagram of various types of humic acid. Notations are the same as in Fig. 5-4.

clearly discriminated from one another, and the B and Rp(1) type areas are included in the B area. The transition from the B area to the A area and further to the bA area indicated the decrease of hydrogen content and the increase of oxygen, but the decrease of nitrogen content was only observed in the diagenesis stage, *i.e.*, the transition from the A area to the bA area.

Considering that nitrogen content in HA is generally very low compared to carbon, hydrogen, and oxygen content, the nitrogen content and N/C ratio seem adequate parameters, and the H″-N″-O″ diagram may not be so useful.

C′-H′-O′ diagram of foreign humic acids

The HAs reported by Kononova (1966), Orlov (1985) and Schnitzer and associates (Matsuda and Schnitzer, 1972; Khan and Schnitzer, 1972; Ortiz de Serra and Schnitzer, 1973; Schnitzer and Skinner, 1974; and

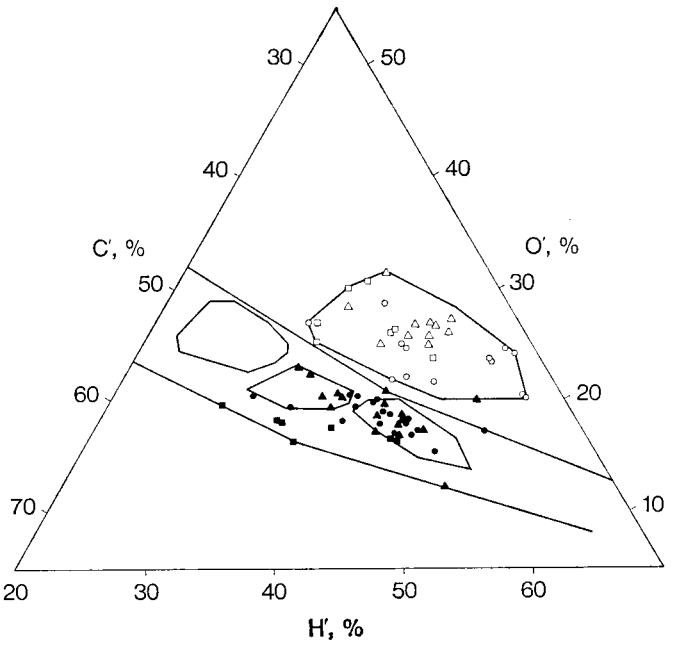


Fig. 5-9. C'-H'-O' diagram of humic acids and fulvic acids reported by Kononova, Orlov and Schnitzer. Notations are the same as in Fig. 5-8.

The FAs prepared by Arai and Kumada (1983) are plotted in Fig. 5-10. F4 and F5 samples were obtained as follows. The alkaline extract of humus was acidified to pH 1.5 with HCl. The HA fraction was separated by centrifugation. The supernatant was neutralized to pH 7.0 by adding 6 N NaOH and concentrated using a rotary evaporator under reduced pressure. The concentrate was acidified to pH 2 and leached through a cation exchange resin (Dowex HCR-S) column to remove metal cations. The leachate was neutralized with NaOH, concentrated in vacuum, and freeze-dried as a crude sample (F3) which contained a considerable amount of NaCl.

An aqueous FA(F3) solution was placed at the top of a Sephadex G-10 column, eluted with H_2O, and a higher molecular weight (>500) fraction was obtained. The higher molecular weight fraction of FA was concentrated under reduced pressure and freeze-dried (designated as F4). The FA sample was refined by passing through anion and cation resins (IRA-400 and Dowex HCR-S) and designated as F5. Ash content of the F4 samples ranged between 20 to 30%. Almost all of the ash in F4 was considered to consist of sodium ions balanced by acid functional groups. The ash content of the

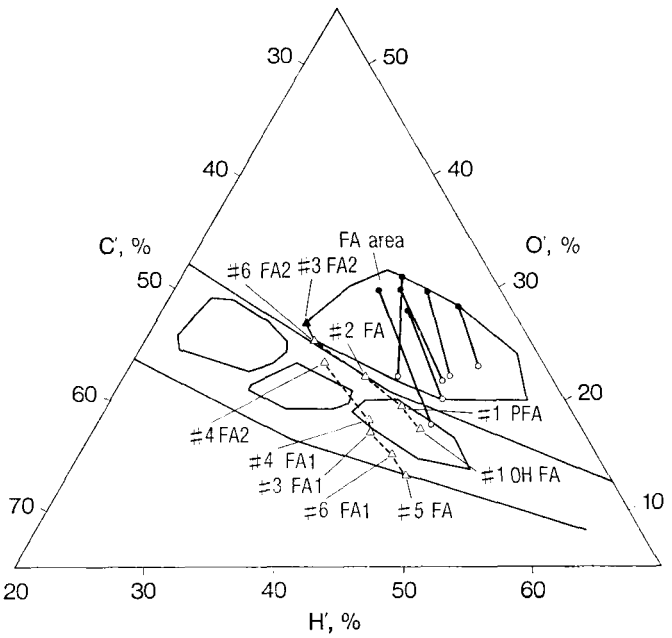


Fig. 5-10. C'-H'-O' diagram of fulvic acids (○: F4; ●: F5) reported by Arai and Kumada (1983) and the PVP adsorbed fractions of fulvic acids (△) by Kumada (1985a).

F5 samples ranged from 5.7 to 0.3%, and about 20% of the FA(F4) sample was lost during the refining procedure.

In Fig. 5-10, the F4 and F5 samples of each FA were linked by straight lines. These lines clearly show that the refining procedure resulted in the decrease of hydrogen and carbon contents and the increase of oxygen content. No significant change was observed in nitrogen content. These results indicate that the FA purification using ion exchange resins caused large losses of the FA and the lost fraction was composed of substances rich in carbon and hydrogen and poor in oxygen. The experiments mentioned here were rather preliminary, and further investigation is needed.

It should be noted, however, that most of the F4 and F5 samples were involved in the area where the FAs reported by Kononova, Orlov, Schnitzer, and Ito (FAs in Fig. 5-2) were distributed. Therefore, this area is tentatively designated as the FA area. The usage of FA "band" was avoided, because no regularity was observed in the distribution of FAs, in contrast to HAs. It is noted that a similar FA area was established in the H/C-O/H diagram.

The C'-H'-O' diagram of the PVP-adsorbed fractions of fulvic acids

The PVP-adsorbed fractions of the FAs described in Chapter 3 are also plotted in Fig. 5-10, and tentatively designated as 'FA.' As mentioned previously, the 10 'FA' samples were divided into two groups and regular changes in the elementary composition (expressed as weight percent) of the 'FA' in each group were observed, that is, with the increase of the carbon content, hydrogen and nitrogen contents increased and oxygen contents decreased.

As seen in Fig. 5-10, the 'FA's of the two groups were respectively arranged in almost a straight line from the inside or vicinity of the B area toward the left edge of the FA area; 2 out of 10 'FA' samples were located at the left edge of the FA area while the rest remained within the HA band.

As the 'FA's dealt with were obtained by an expedient measure, the regular changes in their elementary composition should be verified by further investigation.

5.5. Relationship between Elementary Composition and Optical Properties

The results of linear regression analyses among C%, H/C, O/H, CQ, *RF* or E_6 and $\Delta\log K$ or E_4/E_6 of the HAs in Tables 5-1 and 5-2 are listed in Table 5-3. Highly significant correlations were observed among parameters of elementary composition and optical property, although the linear association between $\Delta\log K$ or E_4/E_6 and other parameters was not so significant.

TABLE 5-3
Correlation coefficients between optical properties and elementary composition.

Data in Table 5-1					
	$\Delta\log K$	C	H/C	O/H	CQ
RF	−0.880	0.906	−0.898	0.844	0.825
$\Delta\log K$		−0.723	0.720	−0.636	−0.648
C			−0.981	0.893	0.846
H/C				−0.929	−0.921
O/H					0.954
Data in Table 5-2					
	E_4/E_6	C	H/C	O/H	CQ
E_6	−0.770	0.899	−0.930	0.869	0.815
E_4/E_6		−0.686	−0.666	−0.511*	−0.474*
C			−0.964	0.815	0.729
H/C				−0.923	−0.879
O/H					0.974

* Indicates significant at 0.5 to 1% level. Balance significant at 0.05% level.

This finding suggests the existence of an essential relation between elementary composition and visible light absorption presumably mediated by chemical configuration.

SUMMARY

1. The deviation of the analytical values on the elementary composition of 5 to 8 HA and FA samples obtained from 2 soil samples by the IHSS method and the same workers was shown.

2. Variability of analytical values on the elementary composition of HAs obtained from soil samples at the same site by similar methods of preparation by different workers was remarkably larger than the case cited in point 1. In general, there were distinct tendencies that, so far as the HAs obtained from soils at the same sampling site were concerned, the higher the carbon content, the lower the hydrogen and nitrogen contents and the higher the oxygen content.

3. In the case of the HAs extracted successively with NaOH, NaF, and $Na_4P_2O_7$ from Rendzina-like soils, the carbon and oxygen contents increased and the hydrogen and nitrogen contents decreased in this order.

4. For the purpose of general comparison of HAs and FAs originating from various soils, it was considered reasonable to express the elementary composition as the atomic number percent of carbon, hydrogen, nitrogen and oxygen, rather than the weight percent of these elements, and to ignore sulfur and phosphor because of their minimal quantities.

5. Carbon, hydrogen, nitrogen, and oxygen contents, and also H/C, N/C, O/C, O/H, and CQ ratios, and H/C-O/C, H/C-O/H, C′-H′-O′, and H″-N″-O″ diagrams were tentatively adopted as parameters and diagrams, respectively, for characterizing the elementary composition of HAs and FAs, and evaluated in relation to the types of HAs.

6. The bA, A, B, and Rp(1) types were discriminated more or less distinctly from each other with respect to carbon, hydrogen and oxygen contents and H/C, O/H, and CQ ratios, but the Rp(1), P_0, Rp(2), and P types could not be discriminated from each other. The nitrogen content and N/C and O/C ratios were not useful as parameters, although the bA type was characterized by lower nitrogen content, lower N/C ratio, and higher O/C ratio than other types.

7. In the H/C-O/C, H/C-O/H, C′-H′-O′, and H″-N″-O″ diagrams, the HAs belonging to the bA type, A type and other types formed separate areas designated as the bA, A, and B areas, respectively. The B and Rp(1) type HAs formed narrow areas separately within the B area.

8. Most HAs reported by Kononova, Orlov, and Schnitzer were dis-

tributed in or near the B and A areas or their intermediate region.

9. It was proposed that the band which comprises almost all the HAs dealt with be called the HA band. Transition from the B area to the A area, and from the A area to the bA area is inferred to imply humification and diagenesis of HAs, respectively.

10. In the H/C-O/H and C′-H′-O′ diagrams, FAs reported by Kononova, Orlov, Schnitzer, and Arai and Kumada constituted a broad area adjacent to the HA band which was designated as the FA area.

11. The PVP-adsorbed fractions of FAs separated by Kumada occupied a special area which was almost completely separated from the FA area and overlapped the B area.

12. In combination with carbon, hydrogen, nitrogen, and oxygen contents (atomic number percent), and H/C, N/C, and O/H ratios, H/C-O/H and C′-H′-O′ diagrams seem recommendable for characterizing the elementary composition of HAs.

13. Highly significant correlations were observed among parameters of elementary composition and optical property.

Chapter 6

Humus Composition of Soils

In 1967, the author and his collaborators (Kumada *et al.*, 1967; Lowe and Kumada, 1984) proposed a novel method of humus composition analysis (Nagoya method), and have investigated many Japanese and foreign soils using the method. The results are described in this chapter.

6.1. Humus Composition Analysis

Nagoya method

1. Weigh soil samples (<2 mm, pulverized in a porcelain mortar and containing less than 100 mg organic C) into 50 ml Erlenmeyer flasks, and add 30 ml extractant (0.1N NaOH). Heat in a boiling water bath or steamer (100°C) for 30 min with shaking the flasks once per 10 min while heating. After heating, add 1 g Na_2SO_4 to the flasks as a coagulating agent, cool in an ice-water bath, and centrifuge at 11,000 rpm (*ca.* $10{,}000 \times g$) for 15 min.

Decant alkaline extract, and wash soil residue twice with 20 ml extractant containing Na_2SO_4 by centrifugation as before. Combine the extract and washings. Acidify the extract with conc. H_2SO_4 (1 ml/100 ml), and let stand for at least 30 min.

2. Transfer soil residue back to the centrifuge tube with 30 ml 0.1M $Na_4P_2O_7$, and treat again as described above using $Na_4P_2O_7$ instead of NaOH.

3. Filter the acidified extracts respectively through filter paper (Toyo Roshi No. 6) into a 100 ml volumetric flask, wash precipitate with H_2SO_4 (1:100) and make the volume of filtrate (FA) to 100 ml with H_2SO_4 (1:100).

Dissolve precipitate (HA) with 0.1N NaOH, collect the solution in a 100 or 250 ml volumetric flask (depending on HA content) and add 0.1N

NaOH to volume. Determine absorption spectrum in the region of 220–700 nm within 2 hr after dissolution.

4. Determine the amounts of HA- and FA-fractions by acid permanganate oxidation according to the procedure described by Simon and Speichermann (1938). This determination should be conducted within 24 hr, especially for HA. Determine organic matter content of original soil sample by the same procedure as used for HA and FA.

5. Expression of the experimental result:

H_T: Total humus; ml of 0.1N $KMnO_4$ consumed by 1 g soil.

H_E: Extracted humus; the sum of 0.1N $KMnO_4$ (ml) consumed by HA and FA of the two extracts per 1 g soil.

H_E/H_T (%): Extracted humus as percent of total humus.

a and b: The amount of HA and FA, respectively, calculated as ml of 0.1N $KMnO_4$ consumed by HA and FA of each extract corresponding to 1 g soil.

PQ: $a \times 100/(a+b)$; percent of HA in extracted humus (HA+FA).

$\Delta \log K$: $\log K_{400} - \log K_{600}$, where K is the optical density at 400 or 600 nm.

RF: $K_{600} \times 1{,}000/c$, where c is ml of 0.1N $KMnO_4$ consumed by 30 ml of HA solution used for determining absorption spectrum.

The above symbols for NaOH and $Na_4P_2O_7$ extracts are differentiated by adding subscripts 1 and 2, respectively, *e.g.*, a_1, a_2, b_1, *etc.*. The humus extracted by the two extractants is regarded as "free" form and "combined" form, respectively.

fHA and fFA: Free HA and FA ratio (%); calculated as $a_1 \times 100/(a_1+a_2)$ and $b_1 \times 100/(b_1+b_2)$.

HA type: HAs are classified into A, B, Rp, P_0, and P ($P_{\pm} \sim P_{+++}$) types as described earlier, and expressed as *e.g.*, Rp•A; the former and the latter indicate NaOH- and $Na_4P_2O_7$-extracted HA, respectively, and the form of expression is called HA combination type.

Comments

The points at issue concerning the separation of HA and FA were mentioned earlier, and the following is complementary.

1. One fault of this method is that the amount of organic matter is expressed as the amount (ml) of 0.1N $KMnO_4$ consumed and not as that of carbon (*e.g.*, mg). Using the conversion rate 0.1N $KMnO_4$ 1 ml=0.45 mg carbon, Kumada and Ohta (1979) proposed adoption of the colorimetric bichromate oxidation proposed by Tatsukawa (1966) instead of permanganate oxidation. The method allows the content of HA and FA to be expressed as

carbon mg per 1 ml sample solution, and *RF* value is calculated as $K_{600}/c' \times 15$, where c is carbon mg per 1 ml HA solution used for determining absorption spectrum. In the analytical results described below, however, the amounts of HA and FA were expressed as a and b, respectively, according to the original method.

The determination of carbon content of HA and FA solution can also be conducted by the Walkley-Black wet combustion method (Allison, 1965) or Kawada's method (1972), but all of these methods should be regarded as proximate analysis. We are obliged to use one of these methods at present until a simple and reliable method which enables determination of organic carbon in solution becomes available.

2. Another fault of permanganate oxidation is that the value for solid sample is often under- or overestimated, as shown in the next paragraph. It has further been experienced that, in the case of fibrous samples such as L and F layers, extremely lower values are obtained than those by the Walkley-Black method. Consequently, it must be concluded that H_T value and H_E/H_T ratio obtained by the Nagoya method are not reliable.

3. In the Nagoya method, FA is merely quantitatively estimated, but its qualitative estimation is desirable. For example, the Ca/Cf (carbon in PVP-adsorbed fraction/total carbon in FA) ratio proposed by Lowe (1980) is recommendable.

4. A single extraction with 0.1N NaOH or a mixture of 0.1N NaOH and 0.1M $Na_4P_2O_7$ can be used instead of successive extraction with 0.1N NaOH and 0.1M $Na_4P_2O_7$. In fact, the analytical results described below were obtained by the three methods of extraction.

For the acid, base-deficient soils predominating in Japan, a single extraction with NaOH can be used, but the mixed extractant or successive extraction is recommended for soils having various pHs.

5. Throughout this book, soil pH means the one determined for a mixture of soil sample and water (1:2.5) using a glass electrode pH meter, except for the values given in Table 6-1.

6.2. Examples of Humus Composition Analysis

In order to clearly explain the Nagoya method, analytical data on the humus composition of 4 Canadian soils (sample Nos. 1-4 in Table 3-1) and 2 Black soils (Lowe and Kumada, 1984) are presented below. The experimental results are shown in Table 6-1.

A comparison of $H_T \times 0.045$ (to convert ml $KMnO_4$ consumed to % soil carbon) with total carbon obtained with a high temperature induction furnace C-analyzer revealed that the $KMnO_4$ method enabled recovery of 73 to 112%

TABLE 6-1
Humus composition analyses by the Nagoya method.

No.	Soil type	H_T	H_E	H_E/H_T	a_1	b_1	PQ_1	RF_1	$\Delta\log K_1$	a_2	b_2	PQ_2	RF_2	$\Delta\log K_2$	fHA	fFA	HA type
1	Orthic Dark Brown (Chernozemic)	31.4	23.6	75	7.20	7.68	48	40	0.687	7.88	0.84	90	129	0.481	48	90	P_0•A
2	Black Solonetz	159	112	71	57.0	34.4	63	68	0.699	19.1	2.78	87	129	0.543	75	93	B•A
3	Orthic Ferro-Humic Podzol	126	118	94	30.7	84.7	27	72	0.590	0.97	1.68	37	137	0.471	97	98	B_+•$A_\pm$
4	Regosolic Static Cryosol	222	156	71	50.5	65.0	44	29	0.795	28.6	11.5	71	53	0.674	64	85	Rp•B
5	Moderately moist Black soil	365	216	59	109	101	52	117	0.497	4.92	1.57	76	113	0.452	96	98	A•A
6	Moderately moist Black soil	185	136	75	69.1	64.1	52	66	0.585	4.39	1.48	75	95	0.497	94	98	B_+•A

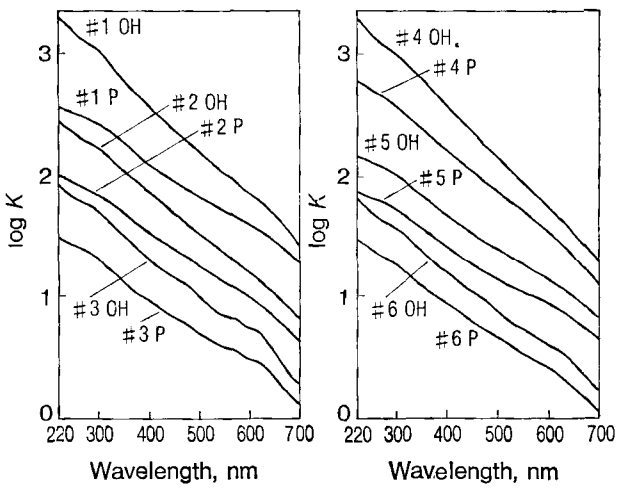


Fig. 6-1. Absorption spectra of humic acids. OH: NaOH extract; P: $Na_4P_2O_7$ extract.

of the amount obtained by the induction furnace method. Agreement between the two methods was not very satisfactory, and it was concluded that the use of $KMnO_4$ may cause significant errors in the estimation of total organic carbon, and hence also in the values for H_E/H_T. Accordingly, the H_E/H_T values cannot serve as an index for characterizing humus composition.

The H_E values ranged from 23.6 to 216 ml 0.1N $KMnO_4$ per g soil. In sample Nos. 3, 5, and 6, having soil pHs of 4.30 to 4.44, both fHA and fFA values were 94 to 98%, that is, a large part of H_E (soluble humus) was obtained by the first NaOH-extraction. In sample Nos. 1, 2, and 5 whose pHs ranged from 5.59 to 6.76, however, the fHA and fFA values were 48 to 75% and 85 to 93%, respectively, indicating that considerable amounts of HA were obtained by the second $Na_4P_2O_7$-extraction, though the amounts of FA were small.

The PQ_1 and PQ_2 values ranged from 27 to 63 and 48 to 97%, respectively, and the former was lower than the latter for the same soil sample. The broad ranges of PQ values indicate that PQ serves as an index for characterizing humus composition. PQ_1 and PQ_2 can be replaced by PQ_{12} ($(a_1+a_2)/H_E \times 100$, %).

Absorption curves of HAs are shown in Fig. 6-1. Sample Nos. 3 OH, 3 P, and 6 OH had Pg absorption. The RF_1 and RF_2 values ranged from 29 to 117 and 53 to 137, respectively; the $\Delta\log K_1$ and $\Delta\log K_2$ values were 0.497 to 0.795 and 0.452 to 0.674. The HAs were assigned as A_+, $A_\pm$, B, B_+, P_0,

and Rp types, and there were observed various combination types, *e.g.*, P_0•A, B•A.

Table 6-1 clearly shows that the RF_1, RF_2, $\Delta\log K_1$, $\Delta\log K_2$, PQ_1, and PQ_2 values and types of HAs vary remarkably with soil sample, and can serve as indices for characterizing humus composition, although in some samples the values and types of HAs for $Na_4P_2O_7$-extracts can be neglected because of their minute amounts.

6.3. Humus Composition of Japanese Soils

Based on the analytical results obtained by the method described above, humus composition of soils of Japan is outlined.

6.3.1. Materials and policy of description

Soils

a. Alpine grassland soils (AGL, n (sample number)=13), Podzols (P, P_D; n=8, P_W; n=2), Dark brown forest soils (dB, n=4), Brown forest soils (B, n=18, yB; n=1) and Red soils (R; n=2). These are all vertically zonal soils distributed in the alpine, subalpine, low mountain, and hilly regions of Central Japan (Kumada and Sato, 1965; Kumada *et al.*, 1966; Kumada and Ohta, 1967; Kumada *et al.*, 1967). In the following description, the Red soils and Brown forest soils are dealt with together (abbreviated RB), because there were only two samples of the former and these could not be discriminated from the latter in humus composition. b. Alpine meadow soils (AM; n=5). c. Rendzina-like soils (n=9). d. Black soils.

Policy of description

Even though the number of soil samples was rather limited, the amount of data was enormous, so the analytical data are arranged and presented in the following manner. (i) H_T and H_E/H_T are excluded. (ii) Humus composition is discussed, emphasizing H_E, PQ_{12}, fHA, fFA, HA types, and the distribution of a and b (amount of HA and FA, respectively) along a soil profile. (iii) The data obtained using three kinds of extractants, NaOH, NaOH+$Na_4P_2O_7$, and NaOH followed by $Na_4P_2O_7$ were dealt with indiscriminately, because there were fundamentally no differences in extracted humus.

6.3.2. Humus composition of A_0 horizon

The A_0 horizon is usually not included in samples for humus composition analysis, but organic matter constituting this horizon seems to be important as one of the sources of soil humus.

In the course of the investigation on the humus composition of forest

soils in Japan, part of the L, F, and H horizon samples were analyzed. The results are first described.

Samples were composed of 4 L, 17 F(F_1, F_2), 8 H, 1 F-H, and 1 H-A layers, 31 in total. The main vegetation of the sampling sites was 5 creeping pines, 5 needle-leaved trees, 3 needle- and broad-leaved trees, and 1 deciduous tree. The pH values of the samples ranged from 3.5 to 4.6, with two exceptions of 5.7 and 5.3 for the F_1 and F_2 layers of a Red soil derived from chlorite-schist, and no relations were observed between pH values and vegetation, soil groups or layers.

Samples were collected from the RB, dB, P_D, and P_W soils, and conveniently divided into the four groups of RB, dB, P_D, and P_W. The H_E (ml of 0.1N $KMnO_4$ consumed by HA and FA per 1 g sample) and PQ_{12} values of each group are plotted in Fig. 6-2. The H_E values of the P_D group tended to be larger than those of the RB group, and the reverse was true for the PQ_{12} values. Vegetation of the P_D group was all creeping pine shrub. An extraordinarily high H_E value of 1,263 ml was found for one sample of the P_W group. The PQ_{12} values of all samples ranged from 50 to 71%, with one exception in each sample belonging to the dB, P_D, and P_W groups.

The fHA and fFA values were determined for 7 samples of the RB

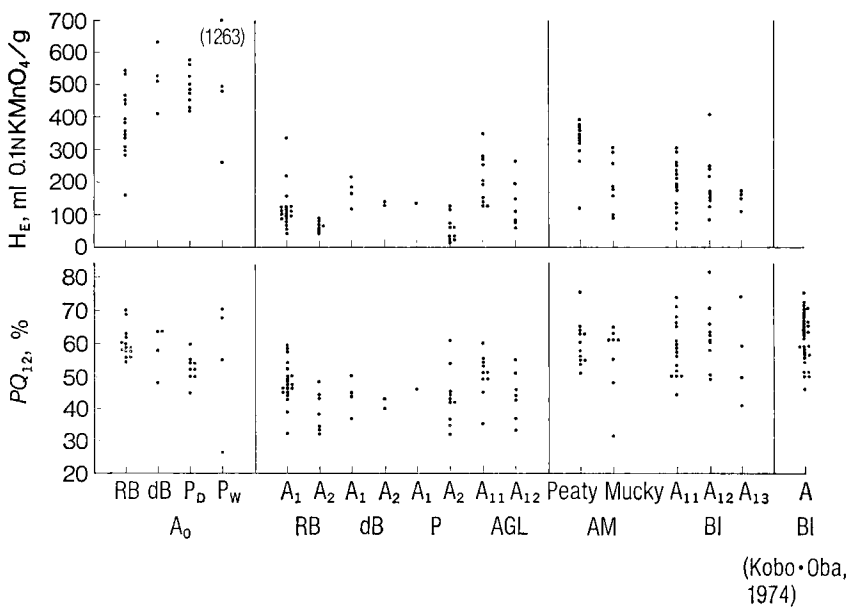


Fig. 6-2. H_E and PQ_{12} values of the A horizons of several soil groups or subgroups in Japan.

TABLE 6-2
Distribution of humic acid types.

HA type	RB group	dB group	P_D group	P_W group
Rp•Rp	L, HA			
Rp•Rp-T	F_1 (2)			
Rp•P_0-T	L, F_1	F_1	F	
Rp•P_0	L, F_1, F_2, FH, H(M)			
Rp•$P_\pm$	F_2			
Rp•B	F_2	F_2		
$Rp_\pm$•$P_\pm$	F_2			
$P_\pm$•$P_\pm$		H(M)		
Rp		H	L, F	F
$Rp_\pm$			F(2), H(M) (2)	
$P_\pm$				H
P_+			H(M)	
P_{++}				F
B				H

group, 3 samples of the dB group, and one sample of the P_D group. Ranges of the fHA and fFA values were 83 to 98% and 90 to 98%, respectively, except for 73 and 86% for the F_1 sample and 84 and 90% for the F_2 sample obtained from the Red soil which had high pH values.

HA types are listed in Table 6-2. (i) NaOH-extracted HAs accounted for a large part of the HAs. All of the RB group belonged to the Rp type; one among them was the $Rp_\pm$ type. In the dB group, there were 3 Rp type, and 1 $P_\pm$ type. In the P_D and P_W groups, out of 12 HAs, 4 belonged to the Rp, 1 to the B and the rest to the $P_{\pm\sim++}$ types. Thus, the main HAs of the A_0 horizon are the Rp or P type. Strictly speaking, the Rp type is Rp(2) type. (ii) In the case of the $Na_4P_2O_7$-extracted HAs, whose amounts were all minute, various types were observed, *e.g.*, Rp-T, P_0-T, P_0. The Rp-T and P_0-T subtypes were found only in the L and F_1 layers, suggesting that tannins disappear in the early stage of humification.

It is premature to make a conclusion on the humus composition of the A_0 horizon because of the limited number of samples, but the results did show: (i) No significant differences could be found among the humus compositions of L, F, and H layers. (ii) Also, no relation could be found between humus composition and vegetation, although the H_E and PQ_{12} values for the RB group tended to be distinguished from those of the P_D group. (iii) The humus composition of the A_0 horizons seemed to be characterized by higher *PQ* values (50–71%), very high fHA and fFA values, and predominance of Rp(2) or P type HAs. (iv) pH values of the samples ranged from 3.5 to 4.6, with two exceptions of 5.7 and 5.3.

The humus composition of the A_0 horizon will be discussed again in Chapter 7.

6.3.3. Forest soils and Alpine grassland soils

pH, degree of base saturation and podzolization

The pH values of A horizons were 3.8 to 5.5 for the RB soils, 3.8 to 5.1 for the dB soils, 4.0 to 4.7 for the P_D soils, 4.0 to 4.5 for the P_W soils and 3.5 to 5.4 for the AGL soils. The pH values increased downward along the soil profiles, the highest value being 5.6. The degree of base saturation of the soil samples was usually less than 10%.

In addition to the soils belonging to the P_D and P_W groups, the distribution of free iron oxides along soil profiles showed a weak podzolization in 2 samples of the dB soils and 3 samples of AGL soils. Thus, the soil samples were acid or strongly acid, most of them remarkably base-unsaturated, and part of them were podzolized.

H_E and PQ values of the A horizons

As mentioned, soil samples were grouped into RB, dB, P(P_D and P_W), and AGL soils. The H_E and PQ_{12} values of the A(A_1, A_{11}) and A_2(A_{12}) horizons of respective groups are plotted in Fig. 6-2. The number sampled of each group was very limited, and the H_E and PQ_{12} values showed rather wide ranges. Therefore, it is very difficult to draw any conclusions, but these trends were observed.

H_E: A_{11} of AGL, A_1 of dB > A_1 of RB, A_{12} of AGL > A_2 of RB, A_2 of P

PQ_{12}: A_{11} of AGL, A_1 of RB, A_1 of dB > A_2 of RB, A_2 of P, A_{12} of AGL

The H_E and PQ_{12} values of the A_1 or A_{11} horizons of all samples were 29 to 344 ml (0.1N $KMnO_4$/g soil) and 27 to 60%, respectively, and the PQ_{12} values of 90% of the samples ranged from 42 to 59%. The H_E and PQ_{12} values of the A_2 horizons of the P soils were 19 to 60 ml and 32 to 61%, respectively.

Only 2 soil samples belonged to the R soils. As this soil group is distributed in warmer regions than the Brown forest soil, it is expected that H_E values are less than those of the latter. The data presented by Kawada (1975b) and Mitsuchi (1985) confirmed this expectation.

Profile distribution of humic acid and fulvic acid

As a means to characterize the humus composition of the respective soil groups or subgroups, distribution diagrams were drawn of the amounts expressed as ml of 0.1N $KMnO_4$ per gram of soil of HA and FA (*a* and *b*) in

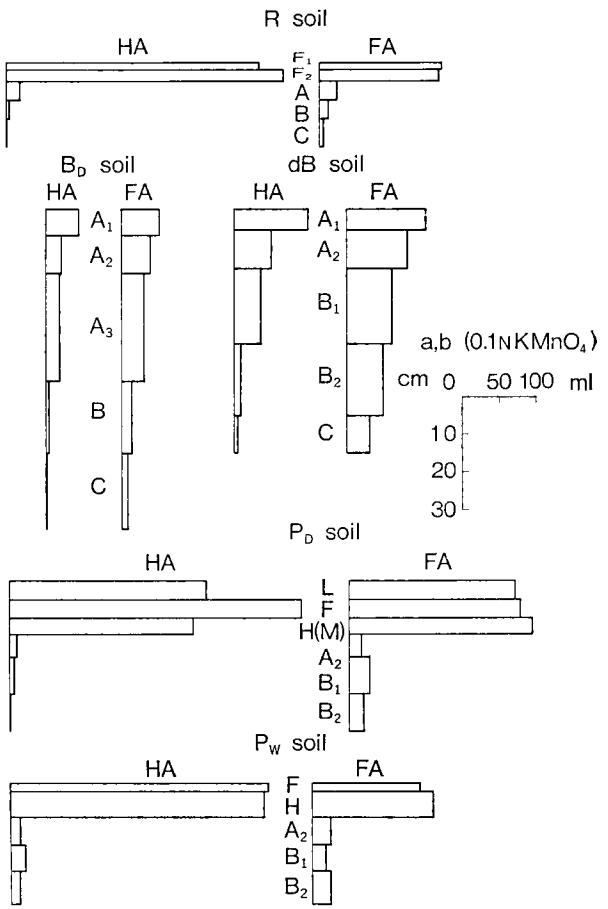
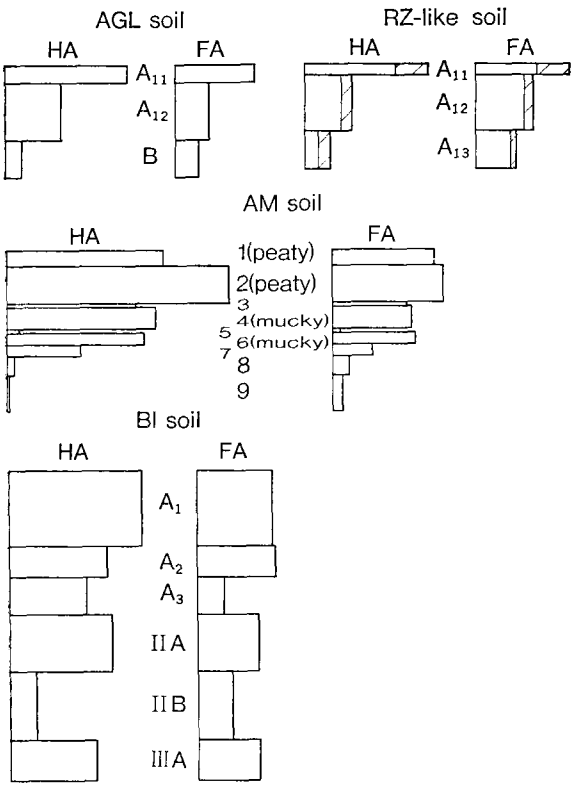


Fig. 6-3. Some examples of the profile distribution of humic acid and fulvic extracted fraction. R soil: Red soil; B_D soil: Moderately moist brown forest soil; AGL soil: Alpine grassland soil; RZ-like soil: Rendzina-like soil; AM

relation to the depth of each horizon (Fig. 6-3). Although the distribution patterns of HA and FA along soil profiles varied considerably between soils, the following differences were qualitatively observed among the soil groups and subgroups.

In RB soils, both HA and FA decreased downward, but the decrease of the former was sharper than that of the latter and consequently the PQ_{12} values lowered along the profile. The dB soils were characterized by the very slow decrease of FA downward through the profile. In Podzols, the amounts of the FA in the B_1 horizon were almost without exception larger than those in the A_2 horizon, and sometimes the amounts of HA too. The distribution



acid in the soils of Japan. The shaded portion of the figure indicates $Na_4P_2O_7$-soil; dB soil: Dark brown forest soil; P_D: Dry podzolic soil; P_W: Wet podzolic soil: Alpine meadow soil; Bl soil: Black soil.

patterns of HA and FA of AGL soils were similar to those of RB soils, though their amounts were larger.

These findings suggest that the graphic representation of the distribution of HA and FA along a soil profile can serve as a means for characterizing humus composition.

fHA and fFA

The fHA and fFA values of the A horizon samples were mostly above 85% and 90%, respectively, indicating that a large part of soluble humus was extracted with NaOH, and the amounts of the $Na_4P_2O_7$-extracted humus

were relatively small. There were tendencies for both values to decrease with depth and those of the B_2 and C horizons were sometimes lower than 50%. Since the lower values were observed for sample whose extractable humus content was very low, it is believed that they were the result of the handling of large amounts of soil samples, rather than that of an increase of combined power between humus and inorganic constituents.

Humic acid types

All the HA types observed in the A and B horizons of the respective groups are listed in Table 6-3.

In RB soils, the NaOH-extracted HAs were of various types, such as Rp, P_0, $P_{\pm\sim+++}$, B, A, and the same was true of $Na_4P_2O_7$-extracted HAs. Most of the A, B, and P_0 types are supposed to have originated from the A type HAs of the relict Black soils which were formed from Akahoya and Aira volcanic ash (Machida and Arai, 1976, 1978; Arai *et al.*, 1984) and widely distributed in the sampling area, the low mountain and hilly zones. If this supposition is permissible, the main HA type of the RB soils may be said to be the P type, followed by the Rp type.

In dB soils, all the HAs were of the P type, with one exception of B_+ type. P type HAs were predominant in P and AGL soils, and the Rp type having Pg absorption followed.

Thus, in summary:

(1) The amounts of extractable humus (H_E values) of respective soil groups showed wide ranges and tended to be arranged in the order of A_{11} of AGL, A_1 of dB > A_1 of RB, A_{12} of AGL > A_{12} of RB > A_2 of P. PQ_{12} values of the A_1 or A_{11} horizons of RB, dB, and AGL soils ranged from 42 to 59%, and those of A_2 horizons of P soils from 32 to 54%.

(2) The distribution patterns of the amounts of HA and FA along the soil profile varied from soil to soil, but RB, dB, P, and AGL soils had peculiar patterns which could be discriminated from one another.

(3) A large part of soluble humus was extracted with NaOH, and the $Na_4P_2O_7$-extracted humus was relatively small.

(4) In RB soils, B, A, and P_0 type HAs were observed in addition to the P and Rp types, but the former was supposed to have originated from the A type HA of relict Black soils. In the dB, P, and AGL soils, P type was predominant, and Rp type having Pg absorption followed.

6.3.4. Alpine meadow soils

Earlier it was mentioned that this soil is composed of a thick humic horizon, a bleached horizon and an iron-illuviated horizon, underlain by impermeable clayey parent materials; it is considered to originally have been

TABLE 6-3
Frequency of humic acid types in soil groups.

A horizon		B horizon		A horizon		B horizon		A horizon		B horizon		A horizon		B horizon	
RB[a]				dB				AGL				Bl			
Rp•B[b]	2	Rp•B	2	$P_{\pm}$•$P_{\pm}$	1	$P_{\pm}$•P_{+}	1	$Rp_{\pm}$•$P_{\pm}$	2	$Rp_{\pm}$•$P_{\#}$	1	A•A	15	B•A	3
Rp•P_0	1	Rp•P_0	1	$P_{\#}$•$P_{\pm}$	2	$P_{\#}$•P_{+}	1	$Rp_{\pm}$•P_{+}	1	$P_{\pm}$•$P_{\pm}$	3	A•P_0	2	$B_{\pm}$•$B_{\pm}$	1
$Rp_{\pm}$•$P_{\pm}$	1	Rp•$P_{\pm}$	3	$P_{\pm}$	1	$P_{\#}$•$P_{\#}$	2	$Rp_{\pm}$•$P_{\#}$	1	$P_{\pm}$•P_{+}	1	A•B	1	A•A	2
P_0•B	1	$Rp_{\pm}$•P_{+}	1	P_{+}	1	$P_{\#}$•$P_{\#}$	2	$Rp_{\pm}$•B	1	P_{+}•$P_{\pm}$	1	A•$P_{\pm}$	1	$A_{\pm}$•B	1
$P_{\pm}$•$P_{\pm}$	2	$Rp_{\pm}$•B	1	$P_{\#}$	1	$P_{\#}$	2	$P_{\pm}$•$P_{\pm}$	4	P_{+}•$P_{\#}$	1	$A_{\pm}$•A	2	$P_{\pm}$•A	1
P_{+}•$P_{\pm}$	2	Rp_{+}•P_{+}	1			B+	1	$P_{\pm}$•P_{+}	1	$P_{\#}$•P_{+}	1	A+•A	1	$P_{\#}$•P_{+}	1
$P_{\#}$•$P_{\pm}$	1	P_0•P_0	1	P				P_{+}•P_{+}	3	$P_{\#}$•$P_{\#}$	1			$P_{\#}$•$P_{\#}$	1
B•A	5	$P_{\pm}$•$P_{\pm}$	2	$P_{\pm}$•$P_{\pm}$	1	Rp_{+}•P_{+}	1	P_{+}•$P_{\#}$	1	$P_{\#}$•$P_{\#}$	1				
$B_{\pm}$•$B_{\pm}$	1	$P_{\pm}$•P_{+}	1	$P_{\#}$•$P_{\#}$	1	Rp_{+}•$P_{\#}$	1	P_{+}•$P_{\#}$	1	Rp$_{\pm}$	1				
A•A	1	P_{+}•$P_{\pm}$	1	Rp_{+}	3	Rp	2	$P_{\#}$•P_{+}	1	$P_{\pm}$	2				
P_0[c]	2	P_{+}•$P_{\#}$	2	$P_{\pm}$	1	$Rp_{\pm}$	1	$P_{\#}$•$P_{\#}$	1	P_{+}	1				
$P_{\pm}$	3	$P_{\#}$•$P_{\#}$	3	P_{+}	2	P_{+}	5	$P_{\pm}$	1	$P_{\#}$	4				
P_{+}	3	B•B	3	$P_{\#}$	5	$P_{\#}$	7	P_{+}	2						
$P_{\#}$	1	B•A	2			$P_{\#}$	1	$P_{\#}$	2						
$B_{\pm}$	1	Rp	1			$B_{\pm}$	1	AM							
B+	1	$P_{\pm}$	4			B$_{\#}$	1	Peaty							
		P_{+}	3					$Rp_{\pm}$	8	P_{+}	5				
		$P_{\#}$	2					P_{+}	4	$P_{\#}$	3				
		$P_{\#}$	2					Mucky		B$_{\#}$	1				
		B	1					$Rp_{\pm}$	3						
		$B_{\pm}$	1					P_{+}	2						
		B+	1					$P_{\#}$	3						
		B$_{\#}$	1					$P_{\#}$	1						
		$A_{\#}$	1					A+	1						

[a] RB: Red soils and Brown forest soils; dB: Dark brown forest soils; P: Podzols; AGL: Alpine grassland soils; AM: Alpine meadow soils; Bl: Black soils.

[b] *e.g.*, Rp•B means HA combination type of HAs obtained with NaOH and $Na_4P_2O_7$, successively.

[c] *e.g.*, P_0 means type of HA extracted with NaOH or NaOH+$Na_4P_2O_7$.

hydromorphic but with fairly good drainage conditions. Five profiles were used for analysis (Kumada and Ohsumi, 1967).

The pH values of the humic A horizons ranged from 3.9 to 4.9, and their degree of base saturation from 0.5 to 6.3%. These pH values tended to rise from A horizon to B and C horizons, with the highest 6.1 and the degree of base saturation did not ascend.

The H_E and PQ values of the peaty and mucky layers of the humic hori-

zons were 121 to 389 ml of 0.1N $KMnO_4$ per g soil (n=12) and 51 to 76%, and 155 to 300 ml (n=6) and 55 to 65%, respectively.

As listed in Table 6-3, the HAs of the peaty layers belonged to P type or Rp type having Pg absorption. In the mucky layers and B horizon, most HAs were of P type, but one sample each of B_{++} type and A_{+} type was observed.

According to a comprehensive study on Peat soils in Hokkaido (Kondo, 1974, 1980), most HAs obtained from peat and muck layers belonged to the Rp type having no Pg absorption. On the contrary, the HAs of AM soils are of the P type or Rp type having Pg absorption. Better drainage conditions have presumably enabled growth of various host plants for *Cenococcum graniforme*, perhaps extremely base-deficient soil environments being beneficial to this growth.

A_{+} and B_{++} type HAs were observed, though only one of each type. A and B type HAs having Pg absorption were sometimes found in dB and P soils, and were not rare in the alpine grassland and meadow soils of Snowdonia, Great Britain, where P type HAs predominated. The genesis of these HAs will be discussed later.

The humus composition of the AM soils was characterized by larger amounts of soluble humus and higher PQ values comparable to those of the A_0 horizons of the forest soils, and the HAs of P type or Rp type having Pg absorption.

Thus the common features of the RB, dB, P, AGL and AM soils are (i) acid or strongly acid, base-unsaturated soils, (ii) the predominance of HAs of P type or Rp type having Pg absorption, despite differences in their morphological characteristics and the distribution patterns of HAs and FAs in soil profiles.

6.3.5. Rendzina-like soils

Although most soils of Japan are acid, it has long been known that alkaline or weakly acid soils derived from coral limestone or marl are distributed in Okinawa Prefecture, and they have been given various local names. In 1956, the author reported that the HA obtained from a Jāgaru at Kunigami, Okinawa Island was assigned as A type (Kumada and Miyasato, 1956). After the return of Okinawa to Japan, these soils were studied by Shinagawa *et al.* (1970) and Kawada (1975b), who also reported the existence of A type HAs in some of them.

On the mainland of Japan, exposures of limestone are observed here and there where alkaline soils have been found (Terao, 1961). The author surveyed limestone areas in Aichi and Mie Prefectures and found alkaline or weakly acid soils originated from limestone; these are tentatively called Rendzina-

like soils. Differing from the soils described previously, these Rendzina-like soils are characterized by the predominance of A and B type HAs. Although the distribution of Rendzina-like soils is quite limited, the fact that their HAs belong to A or B type is very interesting and important from the standpoint of the genesis of HAs, because these soils are surrounded by Red and Yellow soils or Brown forest soils with P or Rp type HAs.

Here, some experimental results on the humus composition of the Rendzina-like soils are outlined. Soil samples were collected around the limestone mines, at the entrance to a stalactite cave, at the top of a limestone outcrop, and on the colluvia derived from limestone. Their broadly ranging pH values seemed to express various stages of maturity. For instance, they may correspond to Protorendzina, Mullartige Rendzina, Mullrendzina or Braunrendzina proposed by Kubiena (Laatsch, 1954). Morphological and pedological studies have not yet been conducted, except for a description of the profiles at the time of sampling. Many of them were presumably immature.

Analytical results according to Kobo and Oba's method

The data on two soil samples are shown in Table 6-4 (Kumada, 1963). Sample No. 1 (pH 8.3) was taken at the entrance to a stalactite cave, where rainwater did not penetrate fully. Sample No. 3 (pH 7.6) was taken at a colluvium of limestone. Annual precipitation at the sampling sites is *ca.* 1,700 mm, but it is inferred that an alkaline pH is maintained because an abundance of calcium ions is continuously supplied from the limestone fragments existing in the A horizon.

According to Kobo and Oba's method (Oba, 1964), the humus was extracted from a mixture of 15 g soil (air-dried, <2 mm) and 150 ml 0.5% NaOH, 0.5% NaF or 0.1M $Na_4P_2O_7$ at 30° for 24 hr. The humus extract was separated from the residues by centrifugation at 7,000 rpm for 15 min after addition of 4.5 g Na_2SO_4. The extract was acidified with conc. H_2SO_4 (1 ml/100 ml), and HA was separated from FA.

The HA- and FA fractions were analyzed according to the Nagoya

TABLE 6-4
Analytical data obtained by Kobo and Oba's method.

Sample	Extractant	*a*	*b*	*PQ*	*RF*	$\Delta \log K$	HA type
No. 1	NaOH	3.3	10.3	24	12.3	0.866	Rp
	NaF	18.6	4.9	79	87.1	0.583	B
	$Na_4P_2O_7$	41.2	12.1	77	120	0.555	A
No. 3	NaOH	44.4	53.5	45	18.3	0.874	Rp
	NaF	19.4	37.6	44	34.0	0.798	Rp
	$Na_4P_2O_7$	46.0	40.8	51	44.1	0.691	B

method (Table 6-4). In sample No. 1, the amount of extracted humus was in the order of $Na_4P_2O_7$, NaF, and NaOH. The *PQ* value of the NaOH-extracted humus was low, and those of the NaF- and $Na_4P_2O_7$-extracted humus were very high. The *RF* and $\Delta\log K$ values of the three HA fractions were remarkably different from one another, and inverse proportionality was observed between the two values. The HAs were Rp, B, and A types, respectively. The amount of extracted humus in sample No. 3 was in the order of NaOH, $Na_4P_2O_7$ and NaF. The *PQ* values were 44 to 51%. Inverse proportionality between the *RF* and $\Delta\log K$ values of the three HAs was also observed, but the differences in the two values were small. The HAs were Rp, Rp, and B types, respectively.

As illustrated here, the quantity and quality of extractable humus of a given soil vary more or less with the kind of extractant. This is the principle of the method of humus composition analysis proposed by Simon (Simon and Speichermann, 1938). Again, the assumption seems appropriate that HA of a soil is an assembly of fractions whose degree of humification and combinative status with polyvalent cations, especially calcium ions, have a multitude of variations, and different extractions draw out a different part of this assembly.

In sample No. 1, NaOH appears to have extracted the HA fraction which was immature and presumably free, *i.e.*, uncombined with polyvalent cations, while $Na_4P_2O_7$ extracted the fraction which was mature and firmly combined with calcium ions. Because of the large differences in the *RF* and $\Delta\log K$ values between the NaOH- and $Na_4P_2O_7$ extracted HAs, the possibility that the two fractions overlapped seems small. It is supposed that NaF withdrew part of the $Na_4P_2O_7$-extracted HA, *i.e.*, the fraction having relatively lower degree of humification and loosely combined with calcium ions. A similar supposition may also be permissible for the FA fractions, although no evidence is available at present.

The differences in HA fractions obtained by NaOH and $Na_4P_2O_7$ in sample No. 3 were small quantitatively and qualitatively, suggesting that the two fractions overlapped to a considerable extent.

These results and discussion led the author to adopt the Nagoya method of successive extraction with NaOH and $Na_4P_2O_7$.

Analysis by the Nagoya method

The analytical data on the pH and humus composition of soil samples used are listed in Table 6-5 (Kumada and Ohta, 1965).

The pH values of sample Nos. 8 and 9 ranged from 4.8 to 5.7. These soils were taken at the summit of Mt. Fujiwara (*ca.* 1,000 m above sea level), and their vegetation was grasses and *Miscanthus sinensis* (Susuki), respectively.

TABLE 6-5
Humus composition of Rendzina-like soils.

Sample No.	Depth (cm)	pH	H_E	a_1	b_1	PQ_1	RF_1	$\Delta\log K_1$	a_2	b_2	PQ_2	RF_2	$\Delta\log K_2$	PQ_{12}	fHA	fFA	HA type
1	0–5	7.7	80.0	30.3	32.9	48	23	0.80	16.4	8.4	66	40	0.70	53	65	80	$Rp{\bullet}P_0/B$
2-1	0–4	7.6	109	31.7	39.3	45	21	0.83	21.9	16.5	57	46	0.65	49	59	70	$Rp{\bullet}P_0$
2-2	4–14	7.5	50.6	9.44	22.5	30	19	0.81	9.7	8.9	52	62	0.56	38	49	72	$Rp{\bullet}P_0$
3	0–4	7.2	183	59.9	69.8	46	19	0.86	33.2	19.8	63	73	0.61	51	64	79	Rp•B
4-1	0–3	7.2	123	36.6	59.6	38	26	0.80	16.5	10.6	61	76	0.58	43	69	85	Rp•B
4-2	3–13	7.0	101	25.3	52.5	35	26	0.79	12.9	10.4	55	89	0.55	38	66	83	Rp•A
5-1	0–10	7.2	98.2	36.6	39.2	48	49	0.75	16.7	5.7	75	118	0.54	54	69	87	B•A
5-2	10–30	6.4	64.6	24.5	24.4	50	75	0.64	13.1	2.6	83	139	0.53	58	65	90	B•A
6-1	0–4	6.1	87.2	29.1	38.7	43	31	0.74	11.8	7.6	61	56	0.63	47	71	84	Rp•B
6-2	4–25	5.5	38.0	9.3	24.2	28	45	0.65	2.6	1.9	42	98	0.49	31	78	93	$P_0{\bullet}A_+$
7-1	0–3	6.1	287	116	106	52	19	0.82	46.3	18.2	72	44	0.65	57	72	85	$Rp{\bullet}P_0$
7-2	3–18	5.8	137	40.5	68.6	37	18	0.81	19.7	8.3	70	83	0.53	44	67	84	Rp•A
7-3	18–28	6.6	85.0	14.8	46.9	24	27	0.78	17.4	5.9	75	172	0.47	38	46	89	Rp•A
8-1	0–12	5.1	100	32.2	59.6	35	32	0.71	5.2	3.2	62	70	0.50	37	86	95	$P_{\pm}{\bullet}P_+$
8-2	12–24	5.7	56.6	12.1	38.4	24	31	0.67	2.8	2.3	54	79	0.44	26	81	94	$P_+{\bullet}P_{++}$
9-1	0–7	4.8	130	43.5	77.0	36	31	0.69	5.9	3.7	61	58	0.55	38	88	95	$P_{\pm}{\bullet}P_+$
9-2	7–30	5.3	44.9	8.5	36.4	19	52	0.54	—	—	—	—	—	—	—	—	$P_+{\bullet}P_{++}$

Annual precipitation was estimated to exceed 2,000 mm. The pH values and humus composition of these samples coincide well with those of the Brown forest soils having an HA combination type of P•P, suggesting that Rendzina-like soils derived from limestone will, in time, become acid Brown forest soils by leaching of calcium ions and acidification in the warm and humid climate of this country. The limestone contains about 5% clay, which serves as the actual parent material.

In Table 6-5, soil samples are arranged in the order of their pH values, since it seems reasonable to suppose that, in soils originating from limestone, pH can serve as an index of their development.

In the first layers (A or A_{11} horizon), humus composition tended to change regularly in samples No. 1 to No. 7. That is, the RF_1 and RF_2 values increased from No. 1 to No. 5, and then decreased, and the reverse was true for $\Delta\log K_1$ and $\Delta\log K_2$ values. The fHA and fFA values increased from No. 1 to No. 7. Sequential change in the combination type of HAs was observed: $Rp\text{•}P_0 \rightarrow Rp\text{•}B \rightarrow B\text{•}A \rightarrow Rp\text{•}B \rightarrow Rp\text{•}P_0$. Furthermore, the RF_{12} values ($=RF_1 \times \mathrm{fHA}/100 + RF_2 \times (1 - \mathrm{fHA}/100)$) showed a change pattern whose apex is No. 5. Thus, so far as the HAs of the first layers are concerned, their progress of humification with the development of soils seems to attain a climax at sample No. 5-1 (pH 7.2), and thereafter retrogresses with soil acidification.

Respective soil profiles showed tendencies of a higher degree of HA humification and lower fHA values in the second layers than in the first layers. This is probably because the amounts of immature organic materials supplied to the second layers are smaller. But regular changes in HA humification degree and fHA values were also observed in the second layers. It should be noticed, however, that sample No. 7-3 (pH 6.6) had an $Na_4P_2O_7$-extracted HA with the highest degree of humification and showed the lowest fHA value. Since the pH value of this layer was higher than that of No. 7-2, this layer may be regarded as the calcium-enriched B horizon.

The data presented here exemplify the transitional changes in humus composition, especially in HAs with the development of Rendzina-like soils derived from limestone. The soils dealt with in this investigation, however, were all considered ones whose pH values could change very rapidly, *i.e.*, calcium ions were easily leached away because of topographic factors. The reasons are as follows.

Sample Nos. 1 and 2 had pH values of 7.7 to 7.5 and their HAs were considered to be immature, as illustrated by the HA combination type of $Rp\text{•}P_0$. On the other hand, Rendzina-like soils are found which have pH values higher than 7 and $Na_4P_2O_7$-extractable A type HAs. In such cases, limestone fragments existing in the A horizon are thought to have always supplied

TABLE 6-6
Analytical data on Kinshozon soil.

Extractant	a	RF	$\Delta \log K$	HA type
NaOH	17.4	28	0.827	Rp
NaF	8.3	73	0.642	B
$Na_4P_2O_7$	22.0	130	0.509	A

enough calcium ions to maintain pH values higher than 7 over a long period, resulting in the formation of A type HAs.

Analytical results of a soil sample (Kinshozan soil) taken around a limestone mine are shown in Table 6-6. The pH value of the soil was 7.7. HAs obtained by successive extraction with 0.1N NaOH, 0.1N NaF, and 0.1M $Na_4P_2O_7$ were Rp, B, and A type, respectively.

These experimental results may indicate that, even if limestone is a common parent material, there are considerable differences in soil forming processes and consequently in humus composition between a case where soils are rapidly acidified and one where soil pH maintains an alkaline value for a long period.

6.3.6. Black soils

Black soils are characterized by a thick black or blackish brown A horizon, predominance of A type HA, frequent presence of buried humic horizons (past A horizons), *etc.*. Some items concerning the humus composition of Black soils are described: (i) Humus composition of A horizon. (ii) Humus composition of B and C horizons. (iii) Humus composition of immature volcanic ash soils. (iv) Changes in humus composition resulting from human interference. The buried humic horizon will be dealt with in Chapter 11.

Humus composition of A horizon

The analytical results of the humus composition of Black (Bl) soils by the Nagoya method were reported by Kumada *et al.* (1967a,b), Ohtsuka (1974a), Ohtsuka and Arai (Kurobokudo Cooperative Research Group, 1984; Wada, 1986), Yoshida *et al.* (1978) and Sakai *et al.* (1982a). From among them, the data on 14 soil samples were chosen as representative of manure virgin soils. The H_E and PO_{12} values of their A_{11}, A_{12} and A_{13} horizons were 70.4 to 300 ml 0.1N $KMnO_4$ per g soil and 50 to 71% (with one exception of 44%) (n; 14), 83.4 to 249 ml (with one exception of 405 ml) and 49 to 71% (with one exception of 82%) (n; 10), and 107 to 164 ml and 41 to 74% (n; 4), respectively. These values are plotted in Fig. 6-2.

Generally, the H_E values decreased from A_{11} horizons to A_{12} horizons,

and further to A_{13} horizons, but the PQ_{12} values did not always decrease correspondingly. As seen in the figure, the H_E values of the B1 soils tended to be comparable to those of the AGL soils, but the PQ_{12} values of the former were higher and comparable to those of the A_0 horizons. The high PQ_{12} value is one of the characteristics of B1 soils.

Most fHA and fFA values were higher than 90%, and 80% levels were few, although it is considered that a large part of the NaOH-extracted humus is in the form of Al-humus complex. The NaOH-extracted HAs were all assigned as A type (n; 22). The $Na_4P_2O_7$-extracted HAs also belonged to A type, with exceptions of 2 P_0 type and one each of $P_\pm$ and B types. Thus, the HA combination type of the B1 soils can be said to be A•A (Table 6-3).

Humus composition of B and C horizons

From the 14 soil samples, 5 were analyzed for their B and C horizons. Part of the data are cited in Table 6-7. The H_E values decreased from B to C horizons sharply. As seen in the table, the PQ_{12}, fHA, and fFA values tended to decrease downward from the B to C horizons, as was the case with Brown forest soils. The HA combination types were diverse, and the presence of types other than A type was noticed, although Rp and P_0 types were absent.

Immature volcanic ash soils

The *RF* and $\Delta\log K$ values of the NaOH and $Na_4P_2O_7$ extracted HAs of extremely immature soil collected near the crater of Mt. Aso (an active volcano on Kyushu) under *Rhododendron kiusianum* (Miyamakirishima) were 25 and 0.84, and 34 and 0.74, respectively, and the straight line linking the positions of the two HAs on the *RF*-$\Delta\log K$ diagram pointed to the P_0 type area (Fig. 6-4). Shinagawa (1962) collected immature A horizon samples

TABLE 6-7
Humus composition of the B and C horizons of Black soils.

No.	Horizon	PQ_{12}	fHA	fFA	HA type
14	B_1	40	55	65	B•A
	B_2	40	12	37	B•A
16	B	41	86	93	A•A
17	B	49	56	77	A•A
	C	17	27	57	B•A
18	B	29	80	89	$P_\pm$•A+
	C	10	54	86	$B_\pm$•$B_\pm$
Ni*	B	53	83	85	$A_\pm$•B
	C_1	20	55	67	$P_{\#}$•$P_{\#}$
	C_2	20	67	79	$P_{\#}$•P+

* Data from Ohtsuka (1979).

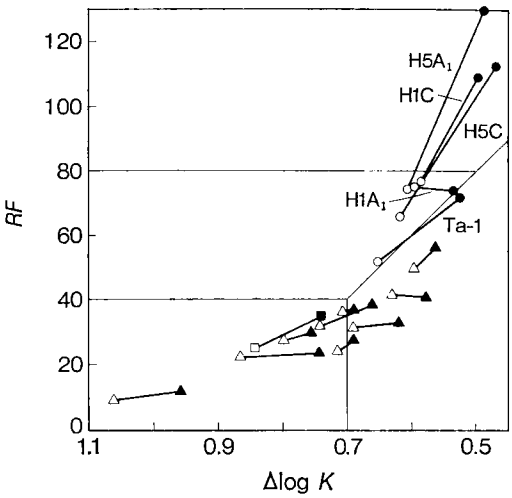


Fig. 6-4. Changes in the *RF* and Δlog *K* values of humic acids of immature Black soils by incubation, and the *RF* and Δlog *K* values of NaOH- and $Na_4P_2O_7$-extracted humic acids of immature Black soils. ○ : NaOH-HA; ● : $Na_4P_2O_7$-HA, Data in Table 6-8 were plotted; □ : NaOH-HA; ■ : $Na_4P_2O_7$-HA for Aso soil; △ : before incubation; ▲ : after incubation (Shinagawa, 1962).

TABLE 6-8
Humus composition of immature Black soils.

Sample	H_E	a_1	b_1	PQ_1	$\Delta\log K_1$	RF_1	a_2	b_2	PQ_2	$\Delta\log K_2$	RF_2	fHA	fFA	HA type
OH-1[a]	49.4	24.9	21.1	54	0.678	40.7	1.4	2.0	41	0.685	—	—	—	P_0—
OH-(1)[a]					0.695	28.9								P_0—
Ta-1[b]	108	63	40	61	0.651	52	3.8	1.6	64	0.519	72	94	96	B•P±
$H1A_1$[b]	127	61.4	60.0	51	0.595	75	2.9	2.9	50	0.534	74	95	95	B•B
H1C[b]	23.0	8.2	13.8	37	0.585	77	0.5	0.5	50	0.496	109	94	97	B•A
$H5A_1$[b]	79.8	46.9	27.5	63	0.598	74	3.7	1.8	67	0.486	130	93	94	B•A
H1C[b]	6.7	2.5	3.5	42	0.620	66	0.3	0.3	50	0.468	113	89	92	B+•A

[a] Ohtsuka (1974a). [b] Kurobokudo Cooperative Research Group (1984).

derived from the volcanic ash which erupted from Mt. Sakurajima in 1914, incubated them for several months, and determined the *RF* and Δlog *K* values of the HAs extracted from the samples before and after incubation. As shown in the figure, the Rp type HAs tended to transform into the P_0 type HAs by incubation. These findings suggest strongly that the transformation of Rp type to P_0 type expresses the early stage of humification in volcanic ash soils. The recent study on the soils of the Krakatau Islands by Shinagawa *et al.* (1986) supports this idea.

The humus composition of the 4 immature volcanic ash soils of known ages is shown in Table 6-8. Sample OH-(1) is the volcanic fallout of Mt. Sakurajima in 1914, and sample OH-1 is the A horizon formed on it. The NaOH-extracted HAs of these samples belong to the P_0 type. The IIA horizon underlying the fallout layer was formed on the volcanic ash layer aged 1779 A.D., and the HA was the A type.

Samples Ta-1, H-1, and H-5 were collected from the A horizons derived from Ta-a layer (a volcanic ash layer erupted from Mt. Tarumae in 1739 A.D.). The NaOH-extracted HAs of these samples were of the B type, and minute amounts of the B, A, or $P_{\pm}$ type HA were found in the $Na_4P_2O_7$ extracts. The NaOH-extracted HAs obtained from the IIA horizons of samples H-1 and H-5 underlying the Ta-a layer were assigned as the A type.

These analytical results support the idea that in the case of volcanic ash soils, the HA successively develops from Rp(1) type to P_0 type, B type, and finally to A type.

Based on these findings as well as the information afforded by Dr. Renzo Kondo (personal communication), 200 to 300 years seem necessary for the formation of A type HA. Here "formation of A type" means that the NaOH-extracted HA is assigned as A type.

Kobo and Oba's study

Using 46 soil samples taken from the A horizons of uncultivated volcanic ash soils (Black soils), Kobo and Oba (1974) studied the relationships between humus composition and parent materials, their degree of weathering, the geographic distribution of the soils and other factors. Part of the results concerning the humus composition itself are cited below.

The vegetation was diverse with grasslands and forests occupying approximately equal sites. It is considered that some forests were established as the result of succession from grasslands and the rest was actually planted forests.

Thirty-two out of 46 soil samples (76%) contained A type HAs, illustrating that HAs of Black soils are characterized by this type. The *PQ* values of the samples ranged from 50 to 75%. As shown in Fig. 6-2, they were comparable to those of the A_{11} horizons of the Black soils described previously.

The HA types of the other 14 soil samples were: 1 Rp type, 3 P_0 type, 5 B and 1 $B_{\pm}$ types, and 4 $P_{\pm}$ and 1 P_{+} types. It seems reasonable to consider that the presence of Rp, P_0, or B type indicates immature Black soils. Furthermore, arid soil environments inferred from their gravelly soil texture and high degree of base saturation may have interrupted the progress of Black soil formation. At the same time, the existence of P type suggests that the soils were in fact Brown forest soils, not Black soils, because Takami and Kubo

(1983) reported examples of Brown forest soils derived from volcanic ash, their HAs being assigned as P type.

Soil having Ap horizon

Soils with Ap horizon are included in the literature cited. They were utilized as pastures, wild grassland for grazing, tea gardens, bamboo forests, and abandoned fields. Presumably the Ap horizons have been ploughed, and soil improving materials such as calcium carbonate and fused rock phosphates, chemical fertilizers, and farmyard manure have been applied. In some cases, the humus composition of the Ap horizons could not be discriminated from that of A horizon, but the former seemed to differ primarily in the following aspects: (i) The a and b values were 50 ml 0.1N $KMnO_4$ per g soil or smaller. When the A_{12} horizons were present, the a_1 or a_1 and b_1 values of the Ap horizons were smaller than those of the A_{12} horizons. (ii) The PQ_1 values were 34 to 44% and lower than those of the A_{12} horizons. Thus, the FA fraction and, even more the HA fraction seemed to be more or less decomposed by cultivation. Most HAs of the Ap horizons remained A type, but in some cases had degraded to B or P_0 type.

In afforested soils, some cases have been observed where the H_E values of the A_{11} horizons were smaller than those of the A_{12} horizons. It is uncertain, however, whether this implies the decomposition of the humus of the A_{11} horizons due to afforestation or that the A_{12} horizons were actually IIA_1 horizons.

As described by Kononova (1975), many investigations have been conducted on the changes in humus composition after cultivation of natural soils. But the influence of human interference in the humus composition of natural soils should be studied further.

In summary, the humus composition of Black soils is characterized by the predominance of the NaOH-extractable A type HAs and high (50 to 75%) PQ values.

6.4. Humus Composition of Foreign Soils

6.4.1. Soil of Great Britain

During his stay in Liverpool, 1966, the author took soil samples of a Podzol at Delamere Forest, a Brown earth at a lowland pasture in North Wales, and several AGL- and AM-like soils in Snowdonia under the guidance of the late Dr. H.M. Hurst of Liverpool University, and samples of two Podzols at Windy Hill and Tyrebagger Forest in the suburbs of Aberdeen, Scotland, under the guidance of Dr. E.A. Fitzpatrick of the University of Aberdeen. Information on these soils is described below.

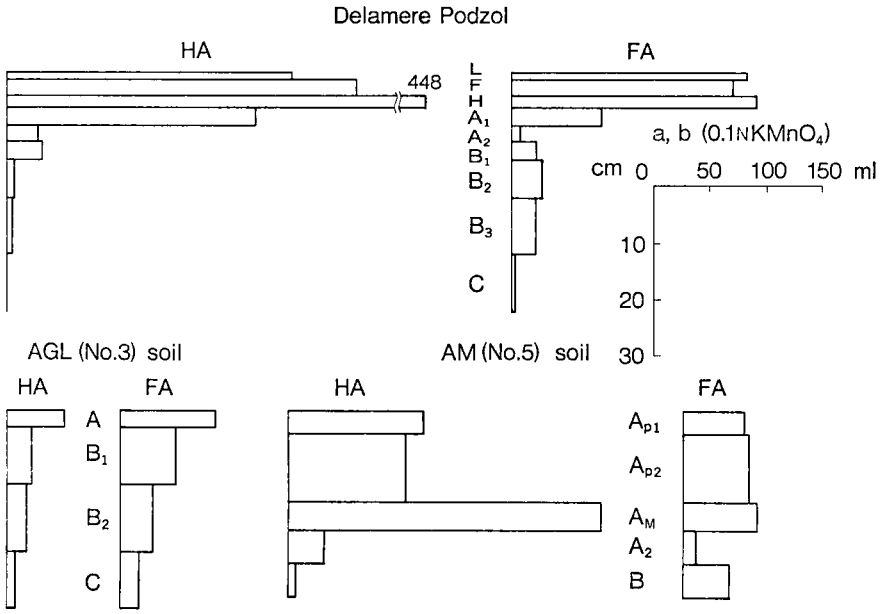


Fig. 6-5. Profile distribution of humic acid and fulvic acid in the soils of Great Britain. AGL: Alpine grassland soil; AM: Alpine meadow soil.

Podzols

The three Podzols corresponded to the P_D soils in Japan and had well developed L, F, and H layers and also A_1 horizon (Kumada, 1967). The profile of Delamere Podzol is shown in Fig. 6-5. The pH values of soil samples ranged from 2.9 to 4.8, were lowest in the H layers, and tended to rise downward through the profiles.

In samples (n=9) of the A_0 horizons, the H_E and PQ_{12} values ranged from 350 to 965 ml 0.1N $KMnO_4$ per g soil, with one exception of 102 ml, and 55 to 68%, respectively, and both fHA and fFA values exceeded 90%. No differences of these parameters were observed among the L, F, and H layers. The HA types and their frequency of the NaOH- and $Na_4P_2O_7$ extracted HAs were 6 Rp type, 1 Rp-T type, 1 $P_{\pm}$ type, and 1 A type; and 1 Rp type, 3 Rp-T type, 2 P_0 type, 1 P_0-T type, 1 B-T type (*RF* 41), and 1 A type, respectively. Thus the Rp type HAs predominated, there was only one $P_{\pm}$ type HA, and the existence of A type HA was noticeable.

The A type HAs were found in the H layer and also in the A_1 horizon of Tyrebagger Podzol, where there was an abundance of charcoal. The charcoal was presumably the result of forest fires. As the HA extracted from the charcoal with 0.1N NaOH exhibited the same shape of absorption curve as

that of soil A type and its *RF* and $\Delta \log K$ values were 134 and 0.508, respectively, the A type HAs observed in the H and A horizons probably originated from the charcoal.

It should be noted that 6 out of 18 absorption curves of HAs determined showed absorption bands suggesting the existence of tannins, and they were all obtained from the L and F layers.

It is evident that the humus composition of the A_0 horizon of the three Podzols resembled that of the A_0 horizons of the acid forest soils in Japan, except for the existence of A type HAs. All three had well developed A_1 horizons, contrary to the Japanese Alpine podzols which lacked this horizon. The H_E values of the B horizons were remarkably larger than those of the A_2 horizons, indicating the significant accumulation of HA and FA. The fHA and fFA values were mostly higher than 90%, although in some samples of the B horizons levels were 80%.

As shown in Table 6-9, the NaOH-extracted HAs were assigned as the P, B or A type, and all of them except for one (A type) had Pg absorption. The A_+ and $B_\pm$ types may be explained as a mixture of the P type and the A type originated from charcoal; this will be discussed later.

Thus the humus composition of the three Podzol profiles was, in principle, not different from that of Japanese Podzols, except for the existence of A and B type HAs.

AGL soils, AM soils, and Brown earth

In Snowdonia, North Wales, grassland soils morphologically resembling

TABLE 6-9
Humic acid combination types of foreign soils.

A horizon	*n*	B horizon	*n*	A horizon	*n*	B horizon	*n*
Podzol				Chernozem			
Great Britain				Czechoslovakia			
$P_\pm$•P_0	4	$P_\pm$~+•$P_\pm$~++	6	Rp•A	1	R_0•$A_\pm$	1
$B_\pm P_0$	1	$B_\pm$•P_0/A/$A_\pm$	3	B•A	2		
A•A	1	$A_\pm$•$A_\pm$	1	Canada			
Czechoslovakia				Rp/P_0/$P_\pm$•A	3		
P_0•nd	1	P_0•P_0/P_+	2	Grey brown podzolic soil			
		P_0•nd	1	Czechoslovakia			
		$P_\pm$•nd	1	Rp•P_0	1	Rp•$A_\pm$	2
Canada				Brown forest soil			
P_+•P_+	2	P_+•P_{++}~+++	2	Czechoslovakia			
A•P_0	1	B_+•$A_\pm$	1	Rp•P_0	1	Rp•$P_\pm$~+	2
A_+•P_+	1					B•P_+	1

n: number of samples; nd: not determined.

the Alpine grassland soils in Japan were widely distributed. Meadow soils resembling the Alpine meadow soils in Japan were observed locally, although the former lacked the volcanic ash and other mineral layers common in the latter. The two kinds of soils are termed here AGL and AM soils for convenience sake. Soils found under the lowland pasture are classified as Brown earths.

The profile distribution of HAs and FAs of these soils is illustrated in Fig. 6-5. Analytical results of the soils of 4 AGL soils, 2 AM soils, and 1 Brown earth are listed in Table 6-10, and outlined below (Ohsumi, 1969).

The pH values of the first layers (A or A_1 horizon) were 4.0 to 4.3, rose downward through the profiles, and the highest was 5.4. The degrees of base saturation of all samples were below *ca.* 10%, and were mostly lower than 5%, with one exception of 26% for the A_1 horizon of the Brown earth. Podzolization was observed in the AM soils. Therefore, these soils were all strongly acid and base-deficient, as was the case of the corresponding soils in Japan.

The H_E and *PQ* values of the A horizons of the AGL soils and the Brown earth were comparable to those of Japanese AGL soils. The H_E values of the peaty horizons of the AM soils were smaller and those of the mucky horizons larger than those of the Japanese AM soils, and their *PQ* values were comparable. As seen in Table 6-10, most HAs belonged to P type, but the A or B type having Pg absorption was not rare.

Compared with corresponding soil groups in Japan, one of the significant, characteristics of the humus composition of the Podzols, AGL and AM soils, and the Brown earth in Great Britain is the relative abundance of the A and B types.

As mentioned, charcoal seems to be the source of the A type in Podzols. However, the facts that the A and B type HAs appeared together with the P type, and that most of them had Pg absorption suggest the transformation from P type to B type, and further to A type. Furthermore, as stated in Chapter 5, the Pg fraction separated from the P type HA was located on the A type area of the *RF*-Δlog *K* diagram, because of the large *RF* and low Δlog *K* values. Accordingly, the HAs assigned as A or B type might, in fact, have been the Pg fraction itself or P type having large *RF* value.

The second difference between the HA types of Great Britain and Japan is that Rp type HAs were absent in the A, B, and C horizons of the former, but present in the latter, presumably reflecting the difference in the maturity of soils. It is considered that the soils in Japan have been continuously eroded and renewed, because of sleep topography and much rainfall and, as a result, immature Rp type HAs have been preserved.

TABLE 6-10
Humus composition of AGL, AM, and BE soils in Great Britain (Ohsumi, 1968).

Soil group[a]	Sample No.	Horizon	pH	DBS[b]	H_E	*RF*	$\Delta \log K$	HA type	*PQ*
AGL	1	A	4.1	10.0	186	36	0.62	P±	49
		B	4.9	5.9	84	91	0.54	A+	49
		C_1	4.8	5.3	4.3	61	0.49	P‡	17
		C_2	5.4	17.0	0.9	44	0.52	P‡	19
	2	A_{11}	4.2	1.1	209	34	0.65	P±	66
		A_{12}	4.1	—	341	46	0.58	P±	54
		B_1	4.6	2.4	117	71	0.55	B+	34
		B_2	4.7	3.1	13.7	46	0.54	B±	33
		C	4.8	3.7	11.8	90	0.48	A‡	13
	3	A	4.3	11.6	141	30	0.65	P±	37
		B_1	4.8	9.2	69.5	56	0.56	P±	30
		B_2	4.9	6.9	43.2	70	0.54	P±	34
		C	5.0	5.3	22.8	63	0.54	P‡	29
	4	A	4.3	3.0	162	35	0.64	P±	53
		B_1	4.7	1.4	78.2	55	0.59	P+	46
		B_2	4.8	1.6	51.6	50	0.50	P+	41
		C	4.9	1.4	27.8	64	0.54	P‡	44
AM	5	A_{11}	4.0	—	173	56	0.65	B±	69
		A_{12}	4.1	1.9	159	78	0.52	B±	65
		A_{13}	3.9	1.4	239	104	0.52	A±	81
		A_2	4.2	1.6	42.5	84	0.60	A+	73
		B	4.4	1.4	45.1	102	0.68	A‡	14
	6	A_{11}	4.2	—	206	78	0.55	B±	50
		A_{12}	4.4	1.5	334	106	0.53	A±	68
BE	7	A_{11}	4.1	26.1	354	43	0.61	P±	56
		A_{12}	4.2	10.3	180	69	0.55	P±	52
		B_1	4.5	3.3	102	87	0.50	A+	43
		B_2	4.6	1.7	38.7	71	0.52	P‡	28
		C	4.7	10.6	6.8	40	0.49	P‡	22

[a] AGL: Alpine grassland soil; AM: Alpine meadow soil; BE: Brown earth.
[b] Degree of base saturation (%).

Despite these differences in HA types, the predominance of P type HA seems to be a common feature of the soils of both Japan and Great Britain.

6.4.2. Soils of Czechoslovakia

The distribution patterns of HAs and FAs of four soil profiles collected in the suburbs of Prague in 1967 are shown in Fig. 6-6. The displayed amounts of HA and FA were 10 times those in Fig. 6-4, and the shaded portion of the figure indicates the $Na_4P_2O_7$-extracted HA and FA. The pH values were

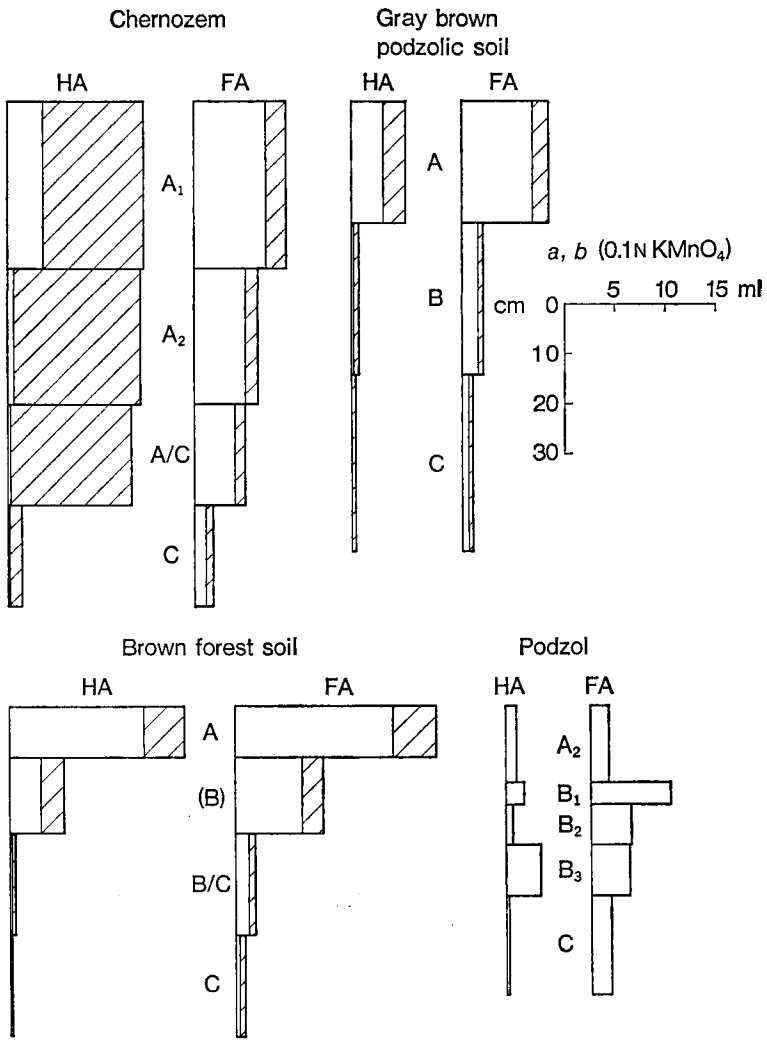


Fig. 6-6. Profile distribution of humic acid and fulvic acid in the soils of Czechoslovakia. The shaded portion of the figure indicates $Na_4P_2O_7$-extracted fraction.

about 8 for the samples of Chernozem, and decreased in the order of Gray brown podzolic soil, Brown forest soil, and Podzol. But the pH values of Podzol were about 5, and higher than those of Podzols of Japan and Great Britain. As seen in Fig. 6-6, the H_E values of these soils were remarkably small compared to those of the soils of Japan.

In the A_1—A/C horizons of Chernozem, the NaOH-extracted fractions were characterized by very low PQ_1 values and Rp or immature B type HAs and, on the contrary, the $Na_4P_2O_7$-extracted fractions by very high PQ_2 values and A type HAs. The fHA and fFA values ranged from 26 to 46% and 78 to 82%, respectively, significantly lower than those of acid soils.

The pH values of Gray brown podzolic soil rose downward through the profile and the fHA and fFA values decreased, suggesting the initiation of acidification or leaching of exchangeable cations. Similarly, the fact that the $Na_4P_2O_7$-extracted HA of the A horizon was assigned as P_0 type may indicate degeneration of the humus of this soil.

The Brown forest soil was different from those of Japan with respect to pH, fHA and fFA values, and the HA combination type was $Rp{\cdot}P_0/P_{\pm}$.

The humus composition of the Podzol was analogous to those of Japan and Great Britain, except that P_0 type HAs were predominant.

6.4.3. Soils of Canada

Analytical data on the humus composition of Canadian soils in each of Chernozemic, Solonetic, Cryosolic, and Podzolic soil samples were described previously. There are additional data on soil samples taken during an inspection tour at the 11th International Congress of Soil Science held in Edmonton in 1978 (Kumada and Ohta, 1979). The humus composition of the three Chernozemic soils including sample #1 in Table 3-1 is summarized as follows. The ranges of the pH, H_E, PQ_1, PQ_2, fHA, and fFA values were from 6.6 to 6.8, 23.6 to 82.9 ml 0.1N $KMnO_4$ per g, 48–54%, 82–90%, 48–51%, and 81–90%, respectively. Although there were only three samples, the ranges of each value were very narrow, except for the H_E values. The NaOH-extracted HAs belonged to Rp, P_0 or $B_{\pm}$ type, but the $Na_4P_2O_7$-extracted HAs were all A type (Table 6-9). Thus, one of the characteristics of Chernozem humus is that about half of the HA is of the $Na_4P_2O_7$-extractable A type. Compared to the Canadian Chernozems, the Czechoslovakian Chernozem had lower PQ_1 and fHA values, presumably because of the higher pH values.

Concerning Podzols, five samples of the Ah, Ae, Bhf, and Bf horizons taken at three sites were analyzed. The data were fragmentary, but indicated a close similarity to those of the Podzols of Japan and Great Britain. A type as well as P type HAs were found, and the existence of charcoal was confirmed in the horizons having the former.

6.4.4. Soils of Thailand

The soil classification system was based on the USDA system and explained by Moorman and Rojanasoonthon (1972). Suzuki *et al.* (1980) carried out a humus composition analysis of a large number of the unculti-

TABLE 6-11

Humus composition of soils of Thailand.

Group 1 (RZ, BF, GM, NCB) (Data from Suzuki *et al.* (1980))

No.	Horizon	pH	H_E	PQ_{12}	fHA	fFA	HA combination type	
13	Ap	8.1	24.4	62	3	61	a	Rp•A
RZ	A_{12}	7.9	29.7	66	4	62	a	Rp•A
14	Ap	7.8	27.4	74	5	66		Rp•P_0
BF	B_1	8.0	8.03	55	6	53	a	Rp•A±
	B_2	8.2	8.86	60	5	51	a	Rp•A
12	Ap	7.8	25.9	69	2	48		Rp•P±
GM	B_1	7.9	8.16	70	3	47		P_0•A
	B_2	8.1	8.51	70	2	42	a	Rp•A
	C	8.1	6.68	65	2	42	a	Rp•A
16	Ap	7.7	34.7	79	5	59	a	Rp•A
BF	A_{12}	7.8	30.8	84	4	75	a	Rp•A
	B_2	7.4	25.2	82	2	74	a	Rp•A
	C	7.8	9.61	76	4	70	a	Rp•A
11	Ap	7.7	31.0	57	13	65	a	Rp•A
GM	A_{12}	7.5	18.1	58	5	55	a	Rp•A
	AB	8.2	11.0	74	2	38	a	Rp•A
	B_t	8.0	2.49	69	3	41	a	Rp•A
6	Ap	7.9	22.9	81	20	80	b	Rp•A
NCB	B_1	7.7	19.2	85	13	85	b	B•A
15	Ap	7.8	26.2	77	29	82	b	Rp•A
BF	A_{12}	7.8	22.3	80	28	83	b	B•A
	B_{2t}	7.4	15.7	82	11	85	b	B•A
Group 2 (NCB)								
3	Ap	6.8	10.6	77	64	85	c	B•A
NCB	B_{3t}	7.4	8.67	83	49	78	d	A•A
	B_{3t}	8.2	6.31	87	12	74	b	Rp•A
7	Ap	7.9	8.07	71	51	82	c	B•A
NCB	AB	7.2	4.39	55	60	80	i	Rp•B±
	B_t	7.0	4.65	50	64	80	i	Rp•B±
8	Ap	6.3	14.0	67	56	82	c	B•A
NCB	B_{2t}	6.5	6.94	51	45	81		P_0•A±
	B_3	6.1	5.62	38	42	60	i	Rp•B±
5	Ap	6.5	20.2	84	69	87	d	A•A±
NCB	A_{12}	6.2	20.1	86	74	87	d	A•A
	B_{21}	6.3	18.6	88	80	85	d	A•A
	B_{22}	7.9	9.0	87	48	82	d	A•A±
9	Ap	5.6	15.5	69	82	90	d	A•A
NCB	A_{12}	6.0	11.9	75	64	87	d	A•A
	B_1	5.5	7.42	78	59	76	d	A•A±
	B_2	5.5	4.42	56	24	81	e	B•A±
4	Ap	5.2	5.18	40	85	92	e	B±•A±
NCB	A_2	4.9	4.55	43	87	92	f	B±•B±
	B_2	4.6	3.26	62	91	84	e	B±•A±
Group 3 (RBL, RYLO, RBLO)								
32	Ap	6.6	9.09	43	70	91	c	B•A±
RBL	B_{21}	6.5	2.95	41	50	66	i	Rp±•B±
	B_{22}	5.8	2.80	39	47	58	i	Rp•B±
35	Ap	6.4	3.90	65	68	89	c	B•A±
RYLO	AB	6.5	1.48	39	70	87	c	B±•A±
	B_3	6.5	1.02	25	69	87		B±•P±
31	Ap	5.9	6.06	42	56	81	c	B•A±
RBL	B_{21}	5.7	10.4	53	72	89	c	B•A±
	B_{22t}	5.4	6.49	37	55	77		Rp•A
28	Ap	6.5	21.7	70	85	91	d	A•A±
RBL	A_3	6.3	14.9	68	83	90	d	A•A±
	B_1	6.2	5.71	51	80	88	d	A±•A
36	A_1	6.0	6.68	66	89	94	d	A•A±
RYLO	B_2	5.8	5.19	54	88	93	e	B•A±

No.	Horizon	pH	H_E	PQ_{12}	fHA	fFA	HA combination type	
29	Ap	5.3	21.8	61	93	96	e	B•A
RBL	B_1	5.5	8.97	51	89	89	e	B•A±
	B_2	5.2	6.61	54	93	93	e	B•A±
33	Ap	6.2	32.9	54	87	90	f	B•B±
RBLO	B_1	5.7	16.7	43	91	90	f	B•B±
	B_2	5.7	4.50	27	70	75	f	B•B±
30	A_1	4.9	22.6	26	91	95	f	B•B
RBL	B_1	5.3	5.81	10	84	82	i	Rp•B±
	B_2	5.3	3.95	9	74	73		Rp•A±
34	A_{11}	5.5	11.9	48	93	97	g	P_0•P_0
RYLO	A_{12}	4.5	8.91	44	95	98	g	P±•P±
	A_{13}	4.8	6.01	40	94	95	h	A±•A±
	B_{2t}	4.8	4.29	34	90	93		B±•B±
Group 4 (GP, RYP, P)								
18	A_{11}	6.7	13.2	61	78	92	c	B•A±
GP	A_{12}	6.1	8.35	63	88	95	d	A•A±
	A_2	5.4	3.79	53	94	97	d	A•A+
	B_1	5.2	3.09	55	93	96	d	A•A+
27	Ap	5.9	13.2	69	89	96	d	A•A±
RYP	A_{12}	5.7	7.83	72	57	86	d	A•A±
	B_t	5.4	2.73	45	88	93	d	A±•A±
19	Ap	6.4	6.72	55	91	96	e	B±•A±
GP	A_2	5.7	3.35	33	85	94	e	B±•A±
	R_{2t}	4.8	2.70	35	93	93	f	B±•B+
23	Ap	5.8	23.1	72	78	90	e	B•A
RYP	A_{12}	5.6	16.7	60	85	92	e	B•A±
	A_2	4.8	11.9	67	96	94	e	B•A±
	B_1	4.8	5.95	62	90	78	e	B•A±
	B_2	4.9	4.68	55	90	75	e	B±•A±
20	Ap	4.9	11.8	37	92	94	e	B±•A±
GP	A_{12}	4.8	11.4	30	88	95	e	B+•A+
	B_1	4.8	5.46	14	84	89		Rp•A±
	B_2	4.7	2.47	14	80	71	i	Rp±•B±
24	A_{11}	5.5	24.6	65	84	93	e	B•A
RYP	A_{12}	4.8	11.0	55	94	96	e	B±•A±
	A_2	4.9	3.63	43	94	97	h	A±•B±
	B_{1t}	5.1	3.04	33	92	94		B±•P±
25	A_{11}	5.6	26.6	47	91	96	g	P±•P±
RYP	A_{12}	5.3	26.1	45	95	97	f	B±•B±
	A_2	5.0	9.01	23	90	96	f	B+•B##
	B_{21}	5.0	5.95	29	85	89	f	B±•B±
	B_{22}	5.3	3.35	16	75	77		Rp•Rp±
26	Ap	4.6	15.7	36	95	96	f	B±•B±
RYP	B_1	4.7	5.20	11	73	84	i	Rp±•B+
	B_2	4.9	3.29	7	64	71	i	Rp+•B##
21	A_1	4.3	16.9	61	97	98	h	A±•B±
GP	A_2	4.3	10.4	58	97	97	h	A±•P±
	B_{2t}	4.7	10.0	49	97	97	h	A±•P±
22	A_{11}	4.7	16.4	75	97	96	h	A•P_0
P	A_{12}	5.2	9.73	79	97	96	h	A±•P±
	A_2	5.0	1.64	70	92	96	h	A±•P±
	B_{2h}	4.9	22.7	40	98	99		B±•P±
	B_{2ig}	5.0	23.7	16	93	98	h	A•P±
	B_{3g}	5.3	13.5	9	85	92	h	A•P_0

RZ: Rendzinas; BF: Brown forest soils; GM: Grumusols; NCB; Noncalcic brown soils; RBL: Reddish brown lateritic soils; RYLO: Reddish yellow latosols; RBLO: Reddish brown latosols; GP: Gray podzolic soils; RYP: Reddish yellow podzolic soils; P: Podzols.

vated and field soils. Soils used included Rendzina (RZ, 1 (number of sample soils)), Grumusols (GM, 2), Brown forest soils (BF, 3), Noncalcic brown soils (NCB, 7), Reddish brown latosol (RBLO, 1), Reddish yellow latosols (RYLO, 3), Reddish brown lateritic soils (RBL, 5), Gray podzolic soils (GP, 4), Reddish yellow podzolic soils (RYP, 5) and Podzol (P, 1). They were utilized as deciduous forest, grasslands, rubber plantations, orchards, and fields (cassava, maize, sorghum, cotton plants, sugarcane, and wheat, *etc.*).

As humus composition is more or less modified by human intervention, the soils were not necessarily appropriate for samples. However, it was considered that chemical fertilizers and soil amendments had been applied only sparingly, so that the effect of such intervention could be largely ignored. For ease of consideration, the soils were divided into the following four groups: 1; RZ, GM, BF, including 1 NCB, 2; NCB, 3; RBL, RYLO, RBLO, and 4; GP, RYP, P. The pH, H_E, PQ_{12}, fHA and fFA values and HA combination type of each group are listed in Table 6-11.

The ranges of pH values of groups 1 to 4 were from 8.1 to 7.4, 8.2 to 4.6, 6.6 to 4.9, and 6.7 to 4.3, respectively. The range of group 1 was narrow, that of group 2 very wide, and those of groups 3 and 4 almost overlapping. In group 4, the changes in pH values among the horizons of a soil having lower pH values than 5 were small, as was true in soils of other groups, but there were trends that the pH values of the horizons of a soil having higher pH values than 5, rose upward along the profile, suggesting a supply of bases from the A_0 horizon.

The H_E values of the uppermost A horizons of groups 1 to 4 ranged from 22.1 to 34.7 ml 0.1N $KMnO_4$ per g, 5.2 to 20.2 ml, 6.7 to 32.9 ml and 6.7 to 26.6 ml, respectively. Even the H_E values of group 1 were far smaller than those of the Japan soils. This may be one of the characteristics of tropical soils, rather than the consumption of humus by agricultural work.

Several examples of the profile distribution of HAs and FAs are shown in Fig. 6-7. With one exception (a Podzol which showed a remarkable accumulation of HA and FA in the B horizon, sample No. 22 in Table 6–11), the amounts of HAs and FAs decreased downward through the profiles. In most soils belonging to group 1, the amounts of HAs and FAs (especially the former) were little changed to a depth of 40 to 50 cm, as illustrated by sample No. 16. Similar patterns were observed in soils belonging to group 2. Generally speaking, however, the distribution patterns of HAs and FAs along the soil profiles were diverse, and their grouping into types as done for the RB, dB, and P soils in Japan was impossible.

As shown in Fig. 6-7 and Table 6-11, the fHA and fFA values as well as the amounts of HAs and FAs varied remarkably with each soil profile, but there were tendencies that the $Na_4P_2O_7$-extractable humus was relatively

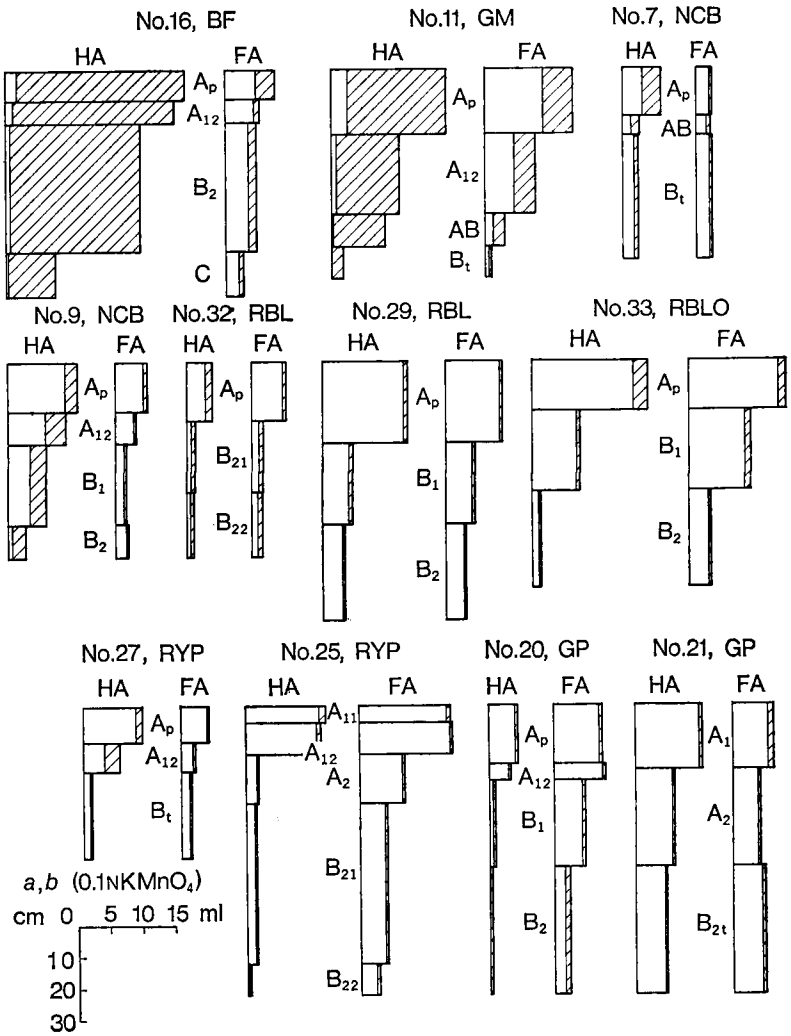


Fig. 6-7. Profile distribution of humic acid and fulvic acid in the soils of Thailand (data from Suzuki *et al.* (1980)). The shaded portion of the figure indicates $Na_4P_2O_7$-extracted fraction.

predominant in horizons having pH values higher than 7, and decreased with acidification.

Grouping of humic acid combination type

According to Table 6-11, the main HA combination types were as follows: a(Rp•A), b(Rp/B•A), c(B•A), d(A•A), e(B•A), f(B•B), g(P•P), h(A•A/B/P). The order of these types from a to h was accompanied by the lowering

of soil pH and the ascent of fHA, and the fHA and pH values enabled discrimination among combination types a, b, c and e (Fig. 6-8). For example, combination types c and e hold B•A in common, but are discriminated from each other by different fHA and pH values.

As seen in the figure, the fHA values of samples having a(Rp•A) type were less than 10%, increased from the b(Rp/B•A) type toward the h (A•A/B/P) type, and attained a level of 90% at the g(P•P) and h types. The fFA values showed similar trends (Table 6-11). The pH values of samples with a and b types were above 7, and tended to go down toward those of the h type. In addition, i(Rp•B) type was observed in the A/B or B horizons with wide pH ranges of groups 2, 3 and 4, and so may be regarded as an immature type. The combination types, such as B•P, P_0•A, *etc.* were observed, but they were left out of consideration because their numbers were very few. Three Rp•A types found in the acid B horizons were also neglected. Except that the i(Rp•B) type was found widely in the A/B and B horizons of groups 2 to 4, the horizons of each soil had the same HA combination type or in some cases two adjacent types.

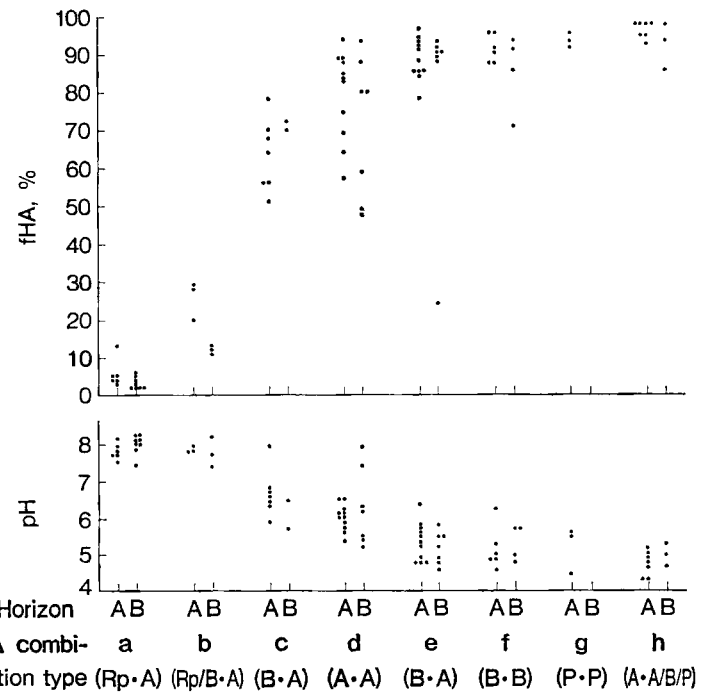


Fig. 6-8. Relationships between fHA or pH value and humic acid combination type (data from Suzuki *et al.* (1980)).

Concerning the relations between groups 1 to 4 and the HA combination types a to h, group 1 is composed of the a and b types, group 2 the c, d, e, and f types with one exceptional b type, and the g type and the g and h types are added to groups 3 and 4, respectively.

The PQ_{12} values of samples by each combination type in the respective groups are shown in Table 6-11. Samples belonging to group 1 and having a(Rp•A) and b(Rp/B•A) types and those in group 2 and having the c(B•A) and d(A•A) types had fairly convergent higher PQ_{12} values, but the rest showed considerably divergent PQ_{12} values.

Thus it is evident that there exist more or less regular relatiouships between the HA combination type and soil pH, fHA, fFA or PQ_{12}.

Incidentally, groups 1 to 4, on the one hand, are respectively composed of many great soil groups, and on the other hand, include plural HA combination types. The relationships between the great soil groups and the HA combination types are as follows:

Group 1: This group is composed of all the samples belonging to the RZ, BF, and GM soils and one sample of the NCB soils; their combination types were a(Rp•A) and b(Rp/B•A). The pH values of the samples ranged from 8.1 to 7.4. Samples of the a type were distinctly discriminated from those of the b type by their lower fHA, fFA, and *PQ* values. In addition, the *RF* values of the NaOH-extracted HAs were 13 to 29 for the a type and 34 to 43 for the b type. Each sample having b type belonged to the BF and NCB soils, and those of the a type belonged to the RZ, BF or GM soils. In other words, the RZ and GM soils had the a type in common, and the BF soils had the a and b types.

Group 2: This group is composed solely of NCB soils. The pH range was wide, 8.2 to 4.6, and there were found the b(Rp•A), c(B•A), d(A•A), e(B•A), f(B•B), and i(Rp•B) types. The pH values of the samples tended to descend in this order, except for the i type. This group illustrates that a great soil group can have various HA combination types.

Group 3: RBL, RYLO, and RBLO soils composed this group. Although samples of the RBLO soil had only the f(B•B) type, the combination types of the RBL and RYLO soils were diverse, and they had three types in common.

Group 4: This group is composed of the GP, RYP, and P soils. Samples of the P soil had the h type, and GP and RYP soils had diverse types with 4 types in common.

It may therefore tentatively be concluded that as far as soil pH, HA combination type, and fHA are adopted as criteria, plural great soil groups can hold one HA combination type in common, a great soil group can have plural HA combination types, and plural great soil groups can have several common

combination types. This conclusion indicates that the great soil groups classified according to morphological characteristics do not always correspond to their humus composition; that is, each great soil group does not necessarily have its own peculiar humus composition. For instance, it is impossible to discriminate Grumusols and Rendzinas from each other with respect to soil pH, HA combination type, and fHA. It seems curious that the Black soils and part of the NCB, RBL, GP, and RYP soils contain the d(A•A) type in common, but that the former should be distinguished from the latter on the basis of other criteria. This problem is left for future investigation.

Frequency of Pg absorption

Although the P type HAs were found exclusively among the soil samples having pH values of 4, other HA types with Pg absorption were often observed in soil samples with wide pH ranges. The frequency of Pg absorption in the HAs is summarized as follows.

Group 1: Only two of $Na_4P_2O_7$-extracted HAs had Pg absorption.

Group 2: Pg absorption was observed in about half of $Na_4P_2O_7$-extracted HAs, and in 3 NaOH-extracted HAs obtained from the soil having the lowest pH values.

Group 3: Seven out of 27 NaOH-extracted HAs and 22 out of 27 $Na_4P_2O_7$-extracted HAs had Pg absorption.

Group 4: Twenty-seven out of 40 NaOH-extracted HAs and 37 out of 40 $Na_4P_2O_7$-HAs had Pg absorption.

There were tendencies that the frequency of Pg absorption was higher in the $Na_4P_2O_7$-extracted HAs than in the NaOH-extracted HAs, and increased from groups 1 to 4, presumably with the increase of soil acidity.

6.5. Generalization of Humic Acid Combination Type

As the series of HA combination types mentioned above were obtained on the basis of the analytical data of a large number of soil samples of Thailand including many great soil groups, it is expected that they have universal validity which can be applicable to the humus composition of soils of countries other than Thailand. To validate this, the humus composition of the soils dealt with previously should be reconsidered.

Soils of Japan

(1) The predominant HA combination type of the RB, dB, P, AGL and AM soils was P•P type and Rp•P type followed, if A and B types often found in the B soils were excluded because of their relict Black soil origin.

The P•P type corresponds to the g(P•P) type. Rp•P type is regarded as the immature P•P type, and called j(Rp•P) type.

(2) The Black soils have A•A type, corresponding to the d(A•A) type.

(3) According to the data listed in Table 6-5, the samples of the Rendzina-like soils can be divided into two groups. In the first group, the pH values ranged from 7.7 to 5.8, the fHA values from 46 to 71%, and the HA combination types were Rp•B, Rp•P_0 Rp•A, and B•A. The last one corresponds to the e type. As P_0 type is close to the Rp or B type, Rp•P_0 type can be included in the i(Rp•B) type. In the second group, the pH and fHA values ranged from 5.7 to 4.8 and 81 to 88%, respectively, and the HAs were assigned as the g(P•P) type. The HA type of Kinshozan soil in Table 6-6 may correspond to the a type.

In Thailand where A and B type HAs are predominant in the A horizons, the i(Rp•B) type was exclusively found in the AB or B horizons. On the other hand, in Rendzina-like soils, the i type was found in the A horizons, suggesting the immaturity of the soils.

Soils of foreign countries

Judging from soil pH, fHA and HA type, it is clear that most of the HA combination types of soil samples dealt with hitherto can be assigned as any of the a–j types. For instance, Chernozem samples of Czechoslovakia and Canada had the b and c types, respectively, and samples #2 and #4 the c and i types, respectively.

Humic acid combination type and soil pH

The series of HA combination types mentioned seemed to be closely related to soil pH; soil pH may be one of the decisive factors which determine HA type. Therefore, the relations between HA combination type and soil pH are considered further.

The appearance of a(Rp•A) and b(Rp/B•A) types can be interpreted as the formation of A type HA firmly combined with calcium ions in soil having pH 8 to 7 and are saturated with exchangeable calcium ions, accompanied by Rp or B type of free form. Also, the d(A•A) type can be regarded as the most stable form of HA under weakly acid soils having plenty of exchangeable calcium ions. RF_{12} values calculated from data listed in Table 6-11 were in the ranges of 90 to 120 for all of a, b and d types with no differences observed among the three.

RF_{12} values for the c(B•A) type were 96 to 58, and significantly smaller than those of the a, b, and d types, despite the fact that pH and fHA values of soils with the c(B•A) type were intermediate between those with a(Rp•A)

and b(Rp/B•A) types and those with d(A•A) types. This seems rather curious, but can be explained:

Among soils having the c(B•A) type, Chernozems in Canada were weakly acid and are considered to be a sort of degraded (leached) Chernozem. On the other hand, the soils in Thailand were all derived from alluvium or colluvium and seem immature. Therefore, the c(B•A) type may be regarded as a degraded or immature example of a, b or d type.

There were tendencies, though not too significant, that soil pH lowered and fHA increased in the order of e(B•A), f(B•B), g(P•P), and h(A•A/B/P) types, suggesting the progress of acidification and leaching of bases in this order. Ranges of RF_{12} values for respective types were 48 to 80, 45 to 66, 38 to 54 and 88 to 97; the order of h type>e type>f type≧g type was rather conspicuous.

As pointed out earlier, the h type found in strongly acid soils was closely related to P type, especially its Pg fraction, and should be expected from the above series. In the strongly acid soils in Japan, Rp type appeared first and then was replaced by P type in due time. Therefore, the j(Rp•P) type is regarded as a forerunner of g(P•P); similarly, the i(Rp•B) type seems to be a forerunner of the c(B•A) or e(B•A) type.

Based on these considerations, P type, B type, and A type represent a stable HA form in strongly acid, acid, and weakly acid to alkaline (pH<8) soils, respectively, although A type is the ultimate form of HA. Considering that P type is probably a composite of Rp type and Pg fraction, this is compatible with the idea that the progress of humification can be expressed as the transformation from Rp type, *via* B type, to A type, and the concept that

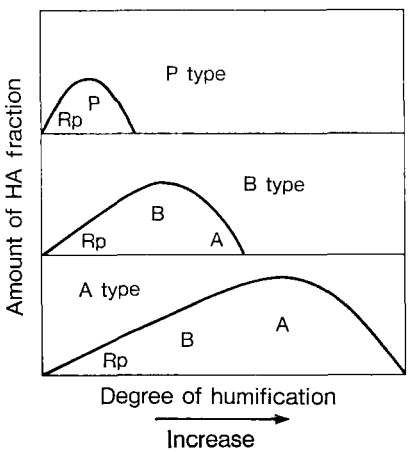


Fig. 6-9. Hypothetical distribution curves of humic acid fractions.

an HA of a soil is an assembly of fractions whose RF and $\Delta\log K$ vary continuously and in inverse proportion.

Thus humus combination types are thought to be systematically understandable by Fig. 6-9, in which the relations between the amount and humification degree of HA fractions of P, B, and A type HAs obtained from soils are shown schematically. The curves represent the hypothetical distributions of HA fractions.

(1) In strongly acid soils, Rp type is mostly replaced by P type, a large part of which is alkali-extractable, giving the g(P•P) type.

(2) In acid soils, Rp type is transformed into B type, most of the B type and the remaining Rp type are extracted by NaOH, and the rest with $Na_4P_2O_7$. In some cases, a small amount of A type combined with calcium ions is formed, which is extracted with $Na_4P_2O_7$. The f(B•B) and e(B•A) types are found.

(3) In weakly acid to alkaline soils, the transformation of HA proceeds from Rp type, *via* B type, to A type, and the last type predominates. In alkaline soils where exchangeable calcium ions and $CaCO_3$ are abundant, Rp or B type and A type are extracted with NaOH and $Na_4P_2O_7$, respectively, giving the HA combination of a(Rp•A) and b(Rp/B•A) types. In weakly acid soils where exchangeable calcium ions are leached to some extent, a mixture of Rp, B and part of A types (assigned as A type overall) is extracted with NaOH, and the remaining A type with $Na_4P_2O_7$, giving the HA combination of d(A•A) type.

SUMMARY

1. Soils of Japan and several foreign countries were analyzed using the Nagoya method of humus composition analysis.

2. Based on the data of Thailand soils, a series of HA combination types was established. The generalized series of HA combination types together with soil pH and fHA as criteria for classification are arranged as follows:

a(Rp•A) type: Soil pH 8.1–7.5; fHA, below 13%.

b(Rp/B•A) type: Soil pH 8–7; fHA, 20–50%. The B type is very similar to the Rp type.

c(B•A) type: Soil pH about 6.5; fHA, 50–80%. Presumably immature or degraded form of the a, b or d type.

d(A•A) type: Soil pH about 6; fHA, 57–98%.

e(B•A) type: Soil pH about 5.5; fHA, 78–96%.

f(B•B) type, g(P•P) type, h(A•A/B/P) type: Soil pH 4–5; fHA, above 85%.

i(Rp•B) type: Soil pH and fHA values have wide ranges. Immature form of the c or e type.

j(Rp•P) type: Soil pH 5–4; fHA above 90%. Immature form of the g type.

The e, f, g, h and j type can be replaced by the HA type of the respective NaOH-extracted HA because of high fHA values; most of them have Pg absorption.

The above-stated division of the HA combination types is admittedly rather expedient, and the criteria cannot be strictly applied. This may be unavoidable, however, because we are dealing with an HA assembly whose degrees of humification and combinative strength with polyvalent bases vary continuously.

3. It may be tentatively concluded that plural great soil groups can have one HA combination type in common, a great soil group can have plural HA combination types, and plural great soil groups can have several common combination types. This conclusion indicates that the great soil groups classified by their morphological characteristics do not always correspond to their humus composition, that is, each great soil group does not necessarily have its own peculiar humus composition.

4. In Rendzina-like soils derived from limestone, regular changes in the humus composition, especially the HA combination type with the lowering of soil pH were observed.

5. Based on the consideration about the relations between HA combination type and soil pH, it was tentatively concluded that P, B, and A types represent stable HA form in strongly acid, acid, and weakly acid to alkaline soils, respectively.

Chapter 7

Analysis of A_0 Horizon

As mentioned in Chapter 1, the transformation processes of plant remains in the A_0 horizon express the early stage of humification of organic debris, and the humified materials can play an important part in soil formation.

For the purpose of deepening our understanding of the A_0 horizon, the author conducted a series of studies about the physically fractionated L, F, and H layers and also the A horizon. Part of the results is outlined.

7.1. Fractionation of A_0 Horizon

The diversity of organic materials constituting the A_0 horizon is exemplified by microscopic observation carried out on L, F, H-A, and A horizons of a yB_B type soil by Ohta and Kumada (1976a): "Organic materials constituting each horizon were composed of various particles which differed in size, shape, color, extent of decay of tissues, amount of adhering mineral particles, *etc.*.

"In the L and F layers, plant remains became smaller in size, darker in color, and mineral particles adhering to them increased with the progress of decomposition. Barks and twigs seemed to be more difficult to decompose compared with leaves. The same was true for the epidermis of leaves, compared with their mesophylls. As for particles smaller than 60 mesh, tissues of plant remains were hardly recognizable, and there were abundant black globular particles, which might have been dropping of soil fauna.

"A large part of plant remains in the H-A and A horizons originated from roots at various stages of decay, and barks, twigs, and black globular particles were few.

"In sand fractions, small amounts of black particles were found. In silt fractions, particles recognizable as being of plant origin were few and brown

amorphous or black globular particles predominated, most of which existed independently apart from mineral particles. On the other hand, clay fractions were greyish brown in color, and a large part of organic matter presumably existed in combination with clay."

Generally speaking, fractionation is one of the effective approaches for

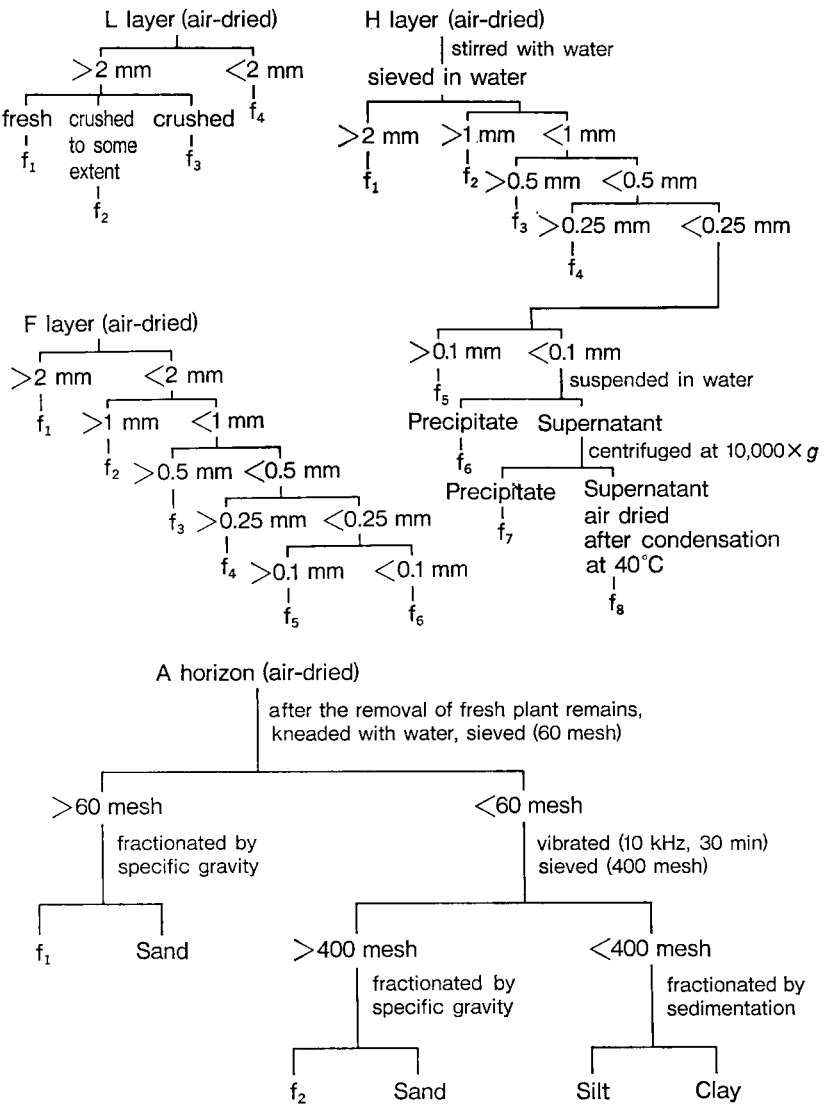


Fig. 7-1. Fractionation scheme of each layer and horizon.

studying complex materials. In the field of soil organic matter (SOM) too, physical fractionation as well as chemical fractionation has been successfully used by several researchers (Wada *et al.*, 1971; Wada, 1972; Takai *et al.*, 1972; Kanazawa *et al.*, 1973), indicating that both methods are useful for analyzing the decomposition and humification process of organic matter in soils.

Following the above study, Ohta and Kumada (1976b, 1977a,b, 1978a) conducted a series of investigations on the fractionated horizons of five forest soils in the Tokai district: Dando I (P_{DIII} type, *Rhododendron*), Owase (B_B type, deciduous broad-leaf trees), Seto (B_B type, deciduous broad-leaf trees and *Pinus densiflora*), Kuragari (B_D type, *Cryptomeria japonica*), and Dando II ($Bl_{D(d)}$ type, *Chamaecyparis obtusa*).

The L, F, H, and A horizon samples were physically fractionated according to the procedures shown in Fig. 7-1.

7.2. Amounts of the Fractions

The weight percentages of each fraction are shown in Table 7-1. The amount of Lf_1 was the largest among the fractions of the L layers, and the sum of this

TABLE 7-1
Weight percentage of fractions from L, F, and H layers.

L layer								
Sample	f_1		f_2		f_3		f_4	
Dando I	51.3		30.3		9.2		9.2	
Seto	44.5		20.0		24.5		29.0	
Owase	35.7		15.7		32.1		16.5	
Dando II			82.2*				17.8	
F layer								
Sample	f_1	f_2	f_3	f_4	f_5	f_6		
Dando I	52.4	15.5	9.7	13.6	5.8	2.9		
Seto	40.5	28.4	14.9	9.5	6.7	Tr.		
Owase	6.4	48.1	17.4	14.2	9.8	4.1		
Dando II	9.0	81.7	4.0	2.7	2.0	0.6		
H layer								
Sample	f_1	f_2	f_3	f_4	f_5	f_6	f_7	f_8
Dando I (H_1)	3.7	5.1	8.4	13.1	15.4	51.3	1.4	1.6
Dando I (H_2)	2.3	0.9	1.1	2.6	6.0	82.4	3.7	1.0
Seto	6.1	5.7	9.2	6.8	5.0	63.9	2.3	1.0
Owase	0.7	2.2	5.0	11.3	4.6	72.1	3.1	1.0

Percent on air-dried basis.
* $f_1+f_2+f_3$.

fraction and Lf_2 was more than half the total weight of each L layer. In particular, in the L layer of Dando I, the sum of the Lf_1 and Lf_2 fractions accounted for 81.6% of the total weight. This may be due to the resistance of *Rhododendron* leaves to breakdown. In the Seto sample, the percentage of the Lf_4 fraction, which was composed of small fragments of *Pinus densiflora* leaves, was considerably large.

In the F layer, the largest fraction was Ff_1 or Ff_2 in each profile, and the sum of these two fractions accounted for about 50–90% of each F layer. The amount of the Ff_1 fraction was the largest in Dando I and Seto, while the Ff_2 fraction was the largest in Owase and Dando II. These differences in the distribution pattern may be caused by differences in properties of the original plant remains.

The largest fraction in the H layer was, without exception, the Hf_6 which comprised more than half of the total weight. The amounts of other fractions were very small. In particular, the H_2f_6 of Dando I accounted for 82% of the H_2 layer.

These results show that the L layer is mainly composed of rather freshly fallen leaves and twigs retaining their original forms; the F layer is composed of broken fragments which did not keep their original form, and which are larger than 2 mm or are between 1 and 2 mm. The H layer is characterized by the fraction (f_6) consisting of quite humified plant remains with particles smaller than 0.1 mm which precipitated overnight.

The L, F, and H layers are distinguishable from each other by the size of the fraction representing each layer. Also, the distribution patterns of fractions in the L and F layers are different from one another owing to the differences in origin of plant remains, although those in the H layers are almost the same, *i.e.*, only the amount of Hf_6 is, without exception, extraordinarily large. These findings indicate that various plant remains, in spite of their different origins, are converted into Hf_6 with the progress of decomposition, and that there are factors inhibiting the further decomposition of Hf_6 in dry type soils.

As the A horizons were not separated quantitatively, the relative ratios of sand, silt, and clay fractions cannot be discussed.

7.3. Elementary Composition

The data of elementary composition are illustrated for Seto and Dando II in Table 7-2. As shown, ash contents ranged from 2 to 9% within the L layer, from 3 to 43% in the F layer and from 2 to 35% in the H layer, and increased in the order of L, F, and H layers. The amount of ash obviously increased as the particle size decreased among the f_1–f_5 or f_6 of the L, F, and H layers. In

TABLE 7-2a.
Elementary composition of fractions from L, F, and H layers.

Sample	Ash (%)	C (%)	H (%)	N (%)	O (%)	C/N
Seto						
Lf_1	5.79	34.1	46.5	0.63	19.1	54.1
f_2	1.88	34.0	45.5	0.64	19.9	53.1
f_3	7.51	34.4	45.5	0.93	19.2	37.0
f_4	7.83	34.4	45.3	1.09	19.2	31.6
Ff_1	15.8	33.7	43.5	0.93	21.8	36.3
f_2	13.1	34.5	44.6	1.30	19.6	26.5
f_3	16.3	33.5	44.4	1.33	20.7	25.2
f_4	34.2	32.5	44.7	1.30	21.5	25.0
f_5	43.3	33.1	43.3	1.50	22.1	22.1
Hf_1	9.54	34.9	44.1	0.85	20.2	41.1
f_2	8.13	35.3	43.6	0.92	20.2	38.4
f_3	14.3	35.0	43.9	1.10	20.0	31.8
f_4	24.4	32.7	41.6	1.26	24.5	25.9
f_5	32.5	35.0	43.8	1.34	19.9	26.1
f_6	24.5	34.5	44.3	1.45	19.8	23.8
f_7	29.8	31.4	47.3	2.27	19.1	13.8
f_8	26.6	29.7	43.7	3.27	23.4	9.1
Dando II						
Lf_1	7.02	35.1	47.5	0.37	17.0	94.6
f_2	4.02	34.7	47.3	0.53	17.4	65.5
Ff_1	3.42	34.8	43.5	0.66	21.1	52.7
f_2	7.11	37.3	42.2	0.90	19.6	41.4
f_3	9.51	35.9	43.4	0.96	19.8	37.3
f_4	17.8	35.0	43.9	1.20	19.9	29.2
f_5	24.7	35.0	44.3	1.18	19.3	29.6
f_6	28.4	34.9	44.4	1.18	19.5	29.6

Figures for ash were expressed as weight percent (moisture-free basis), and those for C, H, N, and O as atomic percentage.

TABLE 7-2b.
Carbon and nitrogen contents of fractions from A horizons.

Profile	Sample	C (%)	N (%)	C/N
Seto	A-Sand	1.38	0.04	34.5
	A-Silt	7.95	0.32	24.8
	A-Clay	7.26	0.41	17.7
Owase	A-Sand	1.37	0.04	34.3
	A-Silt	16.0	0.81	19.8
	A-Clay	29.0	2.00	14.5

Figures expressed as weight percent (moisture-free basis).

the case of Hf_7 and Hf_8, however, their ash contents were all less than that of Hf_5 or Hf_6.

The carbon content (atomic number ratios) of plant remains ranged from 31.4 to 37.3% except for the especially low values of Hf_8 (28.3 to 29.7%). The amount of carbon tended to increase in the order of L, F, and H layers. Carbon content of the L and F layers of Dando II and Kuragari, the vegetation of which was mainly composed of conifers, was higher than those of the other profiles which had mainly broad-leaf trees for vegetation. The hydrogen content ranged from 41.6 to 48.0%, and was slightly higher in L layers than in F and H layers. The value of Hf_7 was the highest among the fractions of one profile. The hydrogen content was higher in the L layers of Dando II and Kuragari (conifer) than in those of the other profiles (broad-leaf trees). The nitrogen content ranged from 0.34 to 3.38%, and increased remarkably in the order of L, F, and H layers and with decreasing particle size within one layer. The nitrogen content of each layer of Dando II and Kuragari was lower than those of the corresponding layers of the other profiles.

The oxygen content varied from 16.8 to 25.6%. There was no regular trend found except that oxygen levels in the L and F layers of Dando II and Kuragari were lower than those of other profiles, and the value of Hf_7 was the lowest and that of Hf_8 the highest within a single profile.

The C/N ratios ranged from 8.5 to 99.7 and were inversely proportional to nitrogen content. As shown in Fig. 7-2, the C/N ratios in each layer of

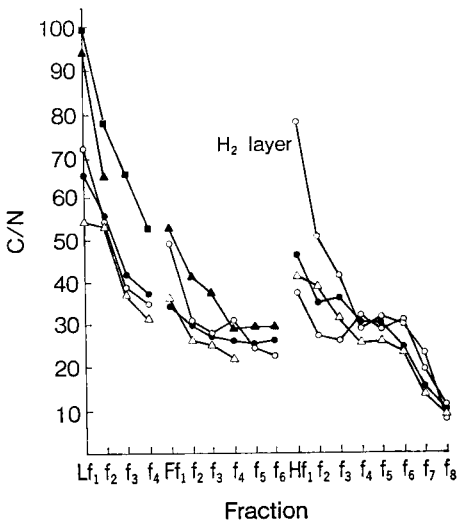


Fig. 7-2. C/N ratios of fractions. ○: Dando I; △: Seto; ●: Owase; ▲: Dando II; ■: Kuragari.

Dando II and Kuragari were higher than in the corresponding layers of other profiles. The fact that the C/N ratio of Hf_8 was about 10 suggests that this fraction contained nitrogen which was largely derived from the bodies of microorganisms. The largest decrease in C/N ratio with particle size was found in L layers. In the F layers, meanwhile, the C/N ratios obviously decreased from f_1 to a value of around 25 or 30 for fraction f_3 or f_4, although no further decrease in C/N ratio was observed with decreasing particle size. Also, in the H layers, the C/N ratio decreased to 25 or 30 for fractions f_3 to f_4, remained nearly constant up to fraction f_6, and decreased further to 10 or so in fractions f_7 to f_8.

It may thus be concluded that organic materials present in the form of litter on the surface of forest soil easily decompose into particles whose C/N ratios ranged between 25 and 30 with diameters between 0.25 mm and 0.5 mm regardless of their origin, and that, depending on the largest amount of Hf_6 within the H layer, further decomposition into materials with C/N ratios lower than 25 or 30 may proceed very slowly in organic layers of dry type soils.

Further, the following trends were found regarding the changes in various parameters which accompanied the disintegration and decomposition of litter: an increase in ash content, a slight increase in carbon content, a slight decrease in hydrogen content and H/C ratio, a remarkable increase in nitrogen content, an obvious decrease in the C/N ratio. Among the values stated above, the change in the C/N ratio is most distinctive, and it is regarded as the most effective index to determine the degradation of litter.

It should be noted that the analytical data obtained for the fractions of L, F, and H layers are, as a whole, quite analogous among the four soil samples used, although the humified litter of conifers has some different properties from those of broad-leaf trees.

As for the sand, silt, and clay fractions of A horizons, there were tendencies that the carbon and nitrogen contents (weight percent) increased and the C/N ratios decreased in the order of sand, silt, and clay fractions. The C/N ratios of clay fractions ranged from 13.8 to 17.7, and were higher for dry type soils than for wet type soils. On the other hand, the C/N ratios (atomic ratio) of Hf_7 and Hf_8 ranged from 13.8 to 23.2 and from 8.5 to 10.0, respectively. There seem to exist some genetic relations between the organic materials contained in clay fractions and those of Hf_7 and Hf_8 fractions. In other words, the Hf_7 and Hf_8 fractions in A_0 horizons are suspected to play an important role in soil formation.

7.4. Humus Composition

Humus composition analysis for the fractionated horizons listed in Table 7-1 was conducted by the Nagoya method using a mixture of NaOH and $Na_4P_2O_7$ as an extractant. The ratios of extractable humus to total humus (C_E/C_T) were calculated as shown in the notes of Table 7-3, where 1 ml of 0.1N $KMnO_4$ was assumed to correspond to 4.5×10^{-4} g of carbon. The data obtained for Seto soil are illustrated in Table 7-3. The data for other samples were analogous to those for Seto soil.

The results obtained for the L, F, and H layers of all samples may be summarized as follows:

(i) The extraction ratios (C_E/C_T) were widely distributed between 30 to 50%. There was no distinct trend shown in this value among soils, layers, or fractions. (ii) The amount of HA (a, ml 0.1N $KMnO_4$ per g sample) ranged from 105 to 547, and there was no trend observed among layers or fractions, although the values for Hf_8 were very low. Furthermore, comparing the values of Lf_1, Ff_1 or Ff_2, and Hf_6, representing the L, F, and H layers, respectively, it was observed that the first and the second were lower than the last.

TABLE 7-3
Humus composition of fractions of L, F, H layers, and A horizon for Seto soil.

Sample	C_E/C_T	a	b	PQ	$\Delta \log K$	RF	Type
Lf_1	43.7	281	234	54.5	0.761	35.3	Rp-T
f_2	42.6	232	262	47.0	0.768	32.6	Rp-T
f_3	41.9	235	259	47.5	0.792	30.0	Rp-T
f_4	42.4	230	268	46.2	0.818	26.1	Rp-T
Ff_1	37.9	181	240	42.9	0.797	30.2	Rp
f_2	41.1	219	270	44.8	0.822	27.0	Rp
f_3	45.2	235	272	46.3	0.820	26.9	Rp
f_4	44.3	219	263	45.5	0.780	34.9	Rp
f_5	42.2	212	244	46.6	0.777	34.5	Rp
Hf_1	35.4	200	203	49.6	0.859	27.6	Rp
f_2	34.2	193	208	48.2	0.870	27.2	Rp
f_3	44.1	265	247	51.8	0.837	29.6	Rp
f_4	46.6	292	250	53.8	0.811	31.4	Rp
f_5	46.7	292	251	53.8	0.772	36.6	Rp±
f_6	47.9	293	261	52.9	0.783	36.7	Rp±
f_7	53.2	305	242	52.2	0.736	33.0	Rp±
f_8	42.5	171	239	41.7	0.800	35.2	Rp±
A-Sand	31.5	4.91	4.61	51.6	0.655	45.2	P+
A-Silt	38.2	29.5	38.0	43.7	0.606	53.7	P+
A-Clay	48.7	27.4	51.1	34.9	0.651	42.4	P+

C_E/C_T: $(a+b) \times 4.5 \times 10^{-4}$(g)/total carbon(%) $\times 100$(%).

(iii) The amount of FA (*b*, ml 0.1N $KMnO_4$ per g sample) ranged from 195 to 632, and most of the values were distributed between 200 and 300. (iv) The *PQ* ratio ranged from 18.2 to 60.6%, and most of the values were distributed between 45 and 60%. Among the *PQ* values of fractions representing each layer, those representing H layers were the highest except in Seto. The low *PQ* values for all the Hf_8 fractions ranging from 18.2 to 41.7% were attributed to the fact that these fractions mainly consisted of water-soluble organic substances. The high *a* and *PQ* values for Hf_6, the amount of which was the largest among fractions of the H layer, might have been related to the difficulty in further disintegration of the fraction.

The *RF* values of HA varied from 18.8 to 46.0. Although the changes in this value were considerably complicated, the values tended to decrease during the initial stage of degradation corresponding to, *e.g.*, the Lf_1–Lf_3 fractions of Seto, which suggests the release and degeneration of colored non-humic substances (maybe tannin-like materials). After this initial stage, the values tended to increase with decreasing particle size and increasing depth. The *RF* values of HAs from the Hf_6 fraction representing the H layer, moreover, were generally higher than those from the fractions representing the L and F layers.

Table 7-3 includes the results for the A horizon. The C_E/C_T values were higher for silt and clay fractions than for sand. The *a* and *b* values increased in the order of sand, silt, and clay fractions, excepting the case of Seto. The order of *PQ* values was sand > silt > clay.

The *RF* and $\Delta \log K$ values of A horizons were evidently larger and lower, respectively, than those of plant remains. And the HAs of fractions obtained from plant remains belonged to the Rp type, while all fractions of A horizons contained P or B type HAs, and were clearly distinguished from plant residual fractions. Thus, it was ascertained that the manner of humification of HAs varied depending on the presence or absence of mineral particles.

The absorption spectra of the HAs are illustrated in Fig. 7-3. All HAs from the fractions of L layers showed shoulders at around 400 and 500 nm, which suggested the presence of tannic materials (Suzuki and Kumada, 1972). The intensity of these shoulders generally decreased with the decrease in particle size. The release or degeneration of tannic substances in the initial stage of decomposition of the litter is obvious, and this may cause the *RF* value to decrease at this stage.

The majority of HAs from the smaller particle fractions of H layers had shoulders near 450, 570, and 620 nm which indicated the presence of the Pg fraction. Without exception, the mineral particle fractions of the A horizons contained the Pg fraction. All HAs of clay fractions, in particular, belonged to the P_+ type.

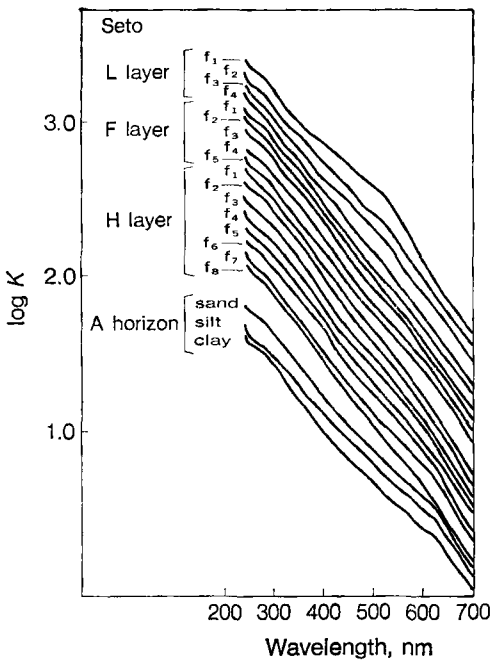


Fig. 7-3. Absorption spectra of humic acids.

According to the results of humus composition analysis, regular changes in humus composition were observed with the gradation of layers and with the decrease in particle size. Most of the HAs from plant residual fractions belonged to the Rp type; HAs from the L layers showed a tannin-like character which disappeared with the progress of decomposition, and HAs from the smaller particle fractions of the H layers contained a Pg fraction. As for HAs from the A horizon, they all belonged to the P or B type and contained a Pg fraction. In addition, it was strongly suggested that the humification process in the mineral horizon was clearly different from that in the organic layer.

Based on the findings obtained on particle size distribution, elementary composition, and humus composition of the fractions of L, F, H, and A horizons, it is stressed that the diversity of SOM does not mean chaos or disorder and, to the contrary, SOM is a well-ordered existence.

7.5. Organic Matter Composition by Waksman's Method

Using Higashiyama soil (yB_B soil, Abemaki (*Quercus variabilis*) and Japanese red pine (*Pinus densiflora*) forest), L and F layers were separated into two

fractions (comparatively fresh Lf_1 and rotten Lf_2), and three fractions (Ff_1; >2 mm, Ff_2; 2-0.2 mm, and Ff_3; <0.2 mm), respectively.

TABLE 7-4
Analytical results on fractions of L and F layers by modified Waksman's method.

Sample	Benzene-ethanol extract	Hot water extract			HCl extract		Cellulose	Residue	Protein
		Total amount	Sugars and starch	Phenolic substance	Total amount	Hemi-celluloses and pectin			
Lf_1	9.26	11.3	(5.55)	(1.81)	15.9	(10.1)	14.0	49.5	0.53
Lf_2	11.7	10.4	(1.00)	(1.69)	18.8	(9.06)	10.1	49.0	1.20
Ff_1	8.53	9.03	(0.98)	(1.22)	17.8	(8.50)	9.25	55.4	1.60
Ff_2	7.82	7.53	(0.70)	(0.79)	16.7	(7.90)	8.03	60.0	2.97
Ff_3	5.99	5.12	(0.54)	(0.38)	19.0	(6.29)	7.72	62.2	3.35

Percent on moisture- and ash-free organic matter basis.

TABLE 7-5
Elementary composition of extracts and residues.

Sample	C	H	N	O	H/C×100	C/N	O/C×100
Benzene-ethanol extract							
Lf_1	22.9	58.6	0.008	6.84	256	2866	29.8
Lf_2	35.6	57.9	0.034	6.49	163	1047	18.2
Ff_1	36.2	57.7	0.059	6.06	159	614	16.7
Ff_2	36.9	56.9	0.078	6.15	154	473	16.6
Ff_3	35.8	57.8	0.106	6.39	162	337	17.9
Hot water extract							
Lf_1	33.5	42.2	0.32	24.0	126	105	71.8
Lf_2	33.1	43.4	0.46	23.0	131	72	69.4
Ff_1	32.3	43.1	0.48	24.2	133	67	74.8
Ff_2	30.9	42.1	0.91	26.1	136	34	84.3
Ff_3	28.8	41.8	0.99	28.4	145	29	98.3
HCl extract							
Lf_1	33.3	38.7	0.78	27.1	116	43	81.4
Lf_2	27.8	37.5	1.16	33.5	135	24	121
Ff_1	24.9	37.5	1.14	36.5	151	22	147
Ff_2	25.7	41.6	1.45	31.2	162	18	121
Ff_3	19.4	39.7	1.32	39.6	205	15	204
Residue							
Lf_1	38.5	41.2	0.95	19.3	107	41	50.1
Lf_2	38.8	41.1	1.42	18.6	106	27	47.9
Ff_1	38.7	38.5	1.39	21.4	100	28	55.3
Ff_2	36.5	36.4	1.30	25.8	100	28	70.7
Ff_3	31.1	31.4	1.34	36.2	101	23	116

Figures expressed as atomic number ratios on moisture- and ash-free basis.

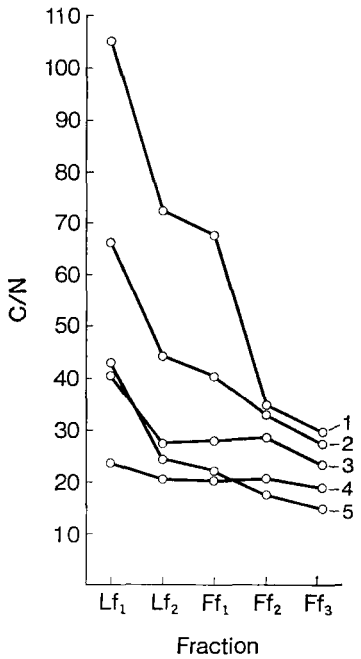


Fig. 7-4. C/N ratios of extracts, residues, and humic acids. 1: hot water extracts; 2: plant remains; 3: residues; 4: humic acids; 5: HCl extracts.

The organic matter composition of the fractions was determined according to modified Waksman's method (Schlichting and Blume, 1966) (Table 7-4). The amounts of residues and protein increased, and those of extracts and components obviously decreased with decreasing particle size from Lf_1 to Ff_3, although there was no distinct trend in the amounts of HCl extract. The amount of benzene-ethanol extract was larger in the Lf_2 than in the Lf_1 fraction; this may be due to the remarkable decreases in amounts of other extractable substances during this stage.

With decreasing particle size, the amounts of sugars and starch, hemicelluloses and pectin, and cellulose decreased in that order. It should be noted that the amount of sugars and starch comprised only a small portion of the hot water extract. Also, phenolic substances comparable to sugars and starch were found in fractions smaller than Lf_2, and they decreased similarly with the decrease in particle size. Hemicelluloses and pectin accounted for only about one half of the HCl extract. It was supposed that hot water extract or HCl extract cannot be represented by sugars and starch or hemicelluloses and pectin, respectively, and that other organic compounds should be taken into consideration.

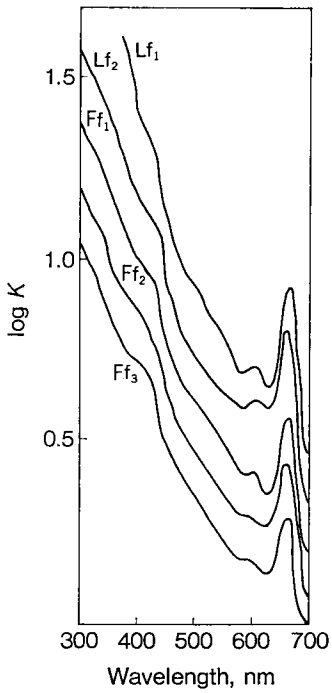


Fig. 7-5. Absorption spectra of benzene-ethanol extracts.

The elementary composition of benzene-ethanol, hot water, and HCl extracts and residues is shown in Table 7-5.

1) The hydrogen content of benzene-ethanol extracts was higher and their nitrogen and oxygen contents were lower than those of other fractions. 2) All extracts and residues contained nitrogen, the amount of which decreased in the order: residues, HCl extracts, hot water extracts, and benzene-ethanol extracts. In the case of residues, however, the increase in nitrogen content was not clear, although that of Lf_1 was the lowest. C/N ratios of all extracts and residues varied noticeably from each other, and tended to decrease with the decrease in particle size (Fig. 7-4). On the other hand, the H/C ratios of hot water and HCl extracts and O/C ratios of all but the benzene-ethanol extract tended to increase.

As shown in Fig. 7-5, absorption spectra of benzene-ethanol extracts distinctly demonstrated the presence of the so-called pheopigments derived from chlorophylls. Their intensities seemed to decrease from Lf_1 to Ff_3.

The data described here indicate the substantial changes which occur during the decomposition and humification process of organic materials composed of litter. However, the results were obtained using only one A_0 horizon sample and further investigation should be conducted using other samples.

Chapter 8

Model Experiments on the Formation of Humic Acids

Since soil formation is a time-consuming and slow process, it is very difficult to analyze it at the actual site where it is taking place. Some kind of model experiment—experimental pedology on SOM—is needed as an effective approach. There are model experiments of various levels, each with its own purpose, and results should be evaluated from the standpoint of to what extent the experiments have succeeded in attaining that purpose.

Let us take the case of HA. For instance, artificial HAs or soil HA-like substances can be easily obtained from carbohydrates, polyphenols and other plant materials by chemical treatments. These experiments may be called a model experiment at the lowest level.

Microbial experiments in which some kinds of microorganisms are grown in media and HA-like substances formed are studied may be a model experiment at the second lowest level. Studies on the formation of HAs during the rotting process of plant remains under laboratory conditions may be called model experiments of a higher level.

The idea that volcanic ash and herbaceous plants, especially *Mithcanthus sinensis* (Susuki in Japanese) are main parent materials of Black soils in Japan seems to be widely accepted. The Black soils are characterized by A type HA. Accordingly, if a mixture of volcanic ash and Susuki plant is incubated and A type HA is obtained, the experiment may be worthy of being called a model experiment of the highest level, because it indicates that we have succeeded in reproducing Black soil in the laboratory.

Many organic substances in nature other than soil are brown in color, soluble in alkali and precipitable by acidification. Similar substances can be prepared by various means in the laboratory. All of them belong to HA according to the definition. It should be verified, however, whether they are

identical to soil HAs or not. How we can distinguish between soil HAs and soil HA-like substances may be the subject of many debates. In the author's opinion, one of the effective criteria for distinguishing between soil HAs and soil HA-like substances is the UV and visible absorption spectrum. However, even if the absorption spectra of the latter are indistinguishable from those of the former, their other properties should be further compared.

It would be interesting and important to evaluate the results obtained hitherto by the numerous model experiments on artificial HAs, but this is too difficult to undertake here. Instead, part of the model experiments conducted by the author are described below.

8.1. Artificial Humic Acids Prepared by Chemical, Enzymatical, and Biological Treatments

In order to contribute to the understanding of the nature and properties and also the formation of HAs, various kinds of artificial HAs were prepared and their properties compared with those of soil HAs (Kumada, 1956a; Kumada and Hotta, 1958). As the criteria for comparison, optical properties (UV- and visible absorption spectrum, $\Delta\log K$ and RF or $E_6^{1\%}$) and also stability were adopted. Stability of HAs was estimated from: (a) Resistance to oxidizing reagent. This was measured by the ratio of amount of 0.1N $KMnO_4$ consumed by HA in alkaline condition (B) to that consumed in acid condition (A), according to the method proposed by Scheffer *et al.* (1950), and given as $B/A \times 100(\%)$. (b) Decolorizability in alkaline solution. This property was estimated from the ratio of optical density at 600 nm of HA solution in 0.1% NaOH determined 14 days after dissolution (K_{14}) to that of the above solution determined immediately after dissolution (K_0), and given as $(K_0 - K_{14})/K_0 \times 100\%$. As shown in Table 8-1, B/A and $(K_0 - K_{14})/K_0$ of soil HAs ranged from 38 to 60% and from 10 to 36%, respectively; proportionality exists between them and they decreased with the humification degree of HA.

Glucose humic acid

Glucose was dissolved in HCl (1:1) or H_2SO_4 (5:95 or 2:8) and the solution was boiled gently. With the lapse of time, the solution became yellow, reddish brown, then blackish brown, and a blackish brown precipitate was formed. The precipitate was separated into 0.5% NaOH-soluble fraction (HA) and insoluble residue. When glucose was treated with boiling 5% NaOH, HA was also obtained, but the yield was very low compared with acid treatments.

As shown in Fig. 8-1, the absorption spectra of glucose (HCl) and glucose (NaOH) HAs were nearly the same, whatever preparative conditions were

TABLE 8-1
Stability of humic acids.

Sample			B/A (%)	$(K_0-K_{14})/K_0$ (%)
Soil HA				
	Tanemori (A type)		38.3	10.5
	Omagari (A type)		38.8	10.0
	Nakajo (Rp type)		49.2	22.0
	Oze (P type)		52.0	25.4
Artificial HA No.		Treatment		
	Glucose	HCl (1:1), 110°, 5 hr	83.4	55.0
	Hydroquinone	5% NaOH, 100°, 5 hr	66.7	53.0
231	Naphthazarin	Thiele and Kettner's method	45.2	21.4
232	β-Naphthol	//	41.9	53.5
237	Alizarin	//	54.4	38.8
242	Alizarin+ Naphthazarin	//	61.6	39.6
272	Tyrosine	Tyrosinase, 30°, 24 hr	37.9	71.2
273	Dopa	//	41.1	66.0
279	Rotten black shells of broad bean		32.8	33.1
246	Charcoal	$KClO_3$·HNO_3	29.4	16.2

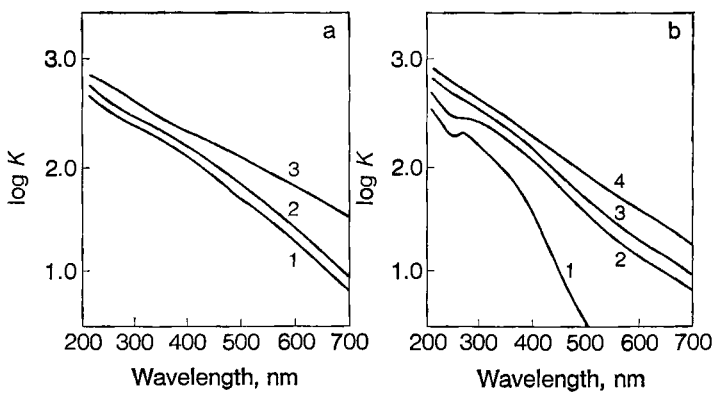


Fig. 8-1. Absorption spectra of artificial humic acids. (a) 1: glucose, 5% NaOH, 100°C, 5 hr; 2: glucose, HCl (1:1), 110°C, 7.5 hr; 3: hydroquinone, 5% NaOH, 100°C, 5 hr. (b) 1: lignin; 2: lignin, HCl (1:1), 110°C, 1 hr; 3: lignin HCl (1:1), 110°C, 10 hr; 4: lignin, HCl (1:1), 110°C, 100 hr.

used, although the light absorption of glucose (NaOH) HA was less than the others. The absorption curves of glucose (H_2SO_4) HAs at the visible region were similar in shape to those of other glucose HAs, but they showed an absorption maximum in the UV region. This absorption maximum tended to decrease, and then to disappear with the duration of boiling or the increase in concentration of the acid.

Hydroquinone humic acid

Hydroquinone HAs were prepared by boiling in 5% NaOH solution for 5 or 20 hr or by allowing the 5% NaOH solution to stand for 20 days at room temperature. Absorption spectra of hydroquinone HAs (Fig. 8-1) were very similar in shape to each other and also to those of A type HAs.

Lignin humic acids

Lignins (alkali-lignins obtained from rice straw and conifer and ethanol lignin from pine) were suspended in HCl (1:1) and boiled for 1 to 100 hr. Lignin HAs were obtained from the residues as in the case of glucose HA by extracting with 0.5% NaOH.

Lignins had an absorption maximum at 275 nm. When they were boiled in HCl (1:1) solution, this maximum disappeared after 1 hr, light absorption at the visible region increased gradually with the duration of boiling, and the curve became increasingly straight, but light absorption at the UV region hardly changed. Thus the inclination of the absorption curve ($\Delta \log K$) decreased with time. The optical properties of HAs obtained by HCl treatment for 100 hr corresponded approximately to B type HA.

From these experimental results, it may be said that the absorption spectra of artificial HAs prepared from glucose and hydroquinone have fairly definite shapes which depend mainly upon their sources, *i.e.*, the kind of organic compound, and that they are little affected by the preparative conditions. On the other hand, the absorption curve of lignin changed remarkably with the duration of boiling in the HCl treatment, and the change seemed analogous to the humification process of HA in soils. Lignin HAs may serve as models of Rp and B types.

As seen in Table 8-1, the B/A and $(K_0-K_{14})/K_0$ values for the hydroquinone and glucose HA were even higher than the highest values for soil HAs, suggesting that they are more or less unstable compared with soil HAs having a low degree of humification.

Thiele and Kettner's artificial humic acids

Thiele and Kettner (1953) prepared artificial HAs from various organic compounds by treating with $K_2S_2O_8$ in 5% NaOH. Absorption spectra of the

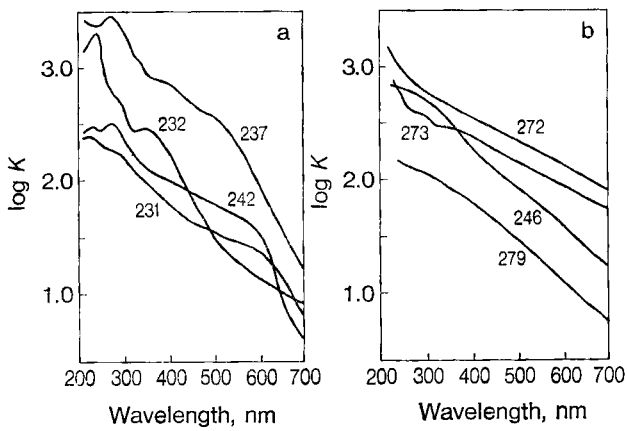


Fig. 8-2. Absorption spectra of Thiele and Kettner's artificial humic acids (a) and melanin and charcoal humic acids (b). Sample Nos. are the same as in Table 8-1.

artificial HAs prepared according to their method are illustrated in Fig. 8-2-a. They had more or less distinct absorption bands in the visible region, and their solid samples were not "brown" in color, although their stability was comparable to that of soil HAs with a low degree of humification. Therefore, it is doubtful that Thiele and Kettner's 'HAs' are worthy of being called artificial HAs. It is presumed that the organic compounds used were too stable for the $K_2S_2O_8$ treatment to result in their oxidative polymerization.

Melanin humic acids

Artificial melanins were prepared as follows. Tyrosine, dihydroxyphenylalanine (dopa) or catechol and potato extract with M/30 phosphate buffer (pH 6.5) were mixed and allowed to stand for 24 hr at 30°C. The mixtures were acidified with HCl and the black residues were recovered, from which HAs were extracted with 0.5% NaOH. HAs were also obtained from well rotted black shells of broad bean, which is said to contain dopa.

As shown in Fig. 8-2-b and Table 8-1, absorption curves of melanin HAs were almost straight in shape, and their stability was comparable to that of soil HAs.

Charcoal humic acids

HAs obtained from charcoal by treatment with $KClO_3$ and HNO_3 (as described in Section 10.2) were very similar to A type HA with respect to absorption spectrum and stability (Fig. 8-2-b and Table 8-1).

TABLE 8-2
Several properties of coal humic acids.

Material	Yield (%)	Ash (%)	$K_{600}^{1\%}$	$\Delta \log K$	$(K_0-K_{14})/K_0$ (%)	B/A (%)
Anthracite	10	3.8	68.6	0.520	6.7	22.8
Bituminous coal	60	5.8	40.9	0.692	19.0	40.2
Brown coal	50	1.5	24.8	0.735	24.2	47.5
Lignite	30	36.6	19.8	0.761	28.6	51.8

HAs were extracted with 0.5% NaOH after pretreatment with $KClO_3$-HNO_3.
Data are shown as oven dried organic matter basis.

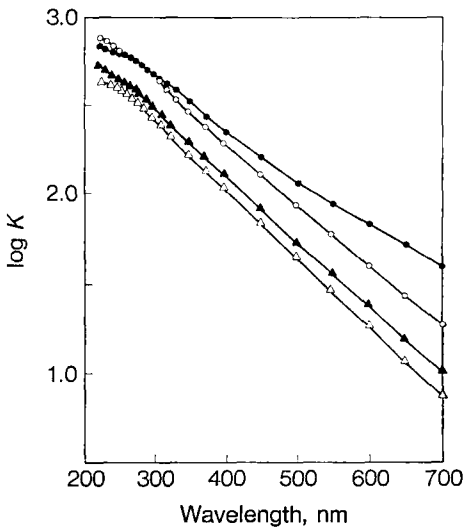


Fig. 8-3. Absorption spectra of coal humic acids. ● : anthracite; ○ : bituminous coal; ▲ : brown coal; △ : lignite.

Coal humic acids

Amakusa anthracite, Joban bituminous coal, Mogami brown coal, and Mogami lignite were pretreated with $KClO_3$ and HNO_3, and HAs were extracted from the residues with 0.5% NaOH. B/A and $(K_0-K_{14})/K_0$ values of coal HAs and their absorption spectra are shown in Table 8-2 and Fig. 8-3, respectively. The absorption spectra of HAs are seen to be similar in shape to those of soil HAs, and the data in Table 8-2 show that anthracite, bituminous coal, brown coal, and lignite HAs corresponded approximately to A, B, Rp, and Rp type HAs, respectively, with respect to their optical properties and stability; these properties changed according to the rank of the original coals.

Microorganism pigment humic acids

Streptomyces pheochromogenes, S. viridochrom and *Botrytis cinerea* were respectively inoculated at 30°C in the medium used by Scheffer *et al.* (1950). After 6-month incubation, a dark reddish brown pigment was produced in the medium of *S. pheochromogenes*, and brownish black pigment in the mycelia of *S. viridochrom* and *Botrytis cinerea*. In *S. pheochromogenes*, the medium, after removal of mycelium by filtration, was acidified by adding HCl, and brownish black precipitate was obtained. The precipitate and the mycelia of the other two strains were air-dried and pulverized. From these materials, HA-like substances were extracted by the following procedure. Each of these materials was suspended in 0.5% NaOH, boiled for 30 min, and the suspension was diluted with water to give a concentration of 0.1% NaOH. The diluted suspension was ultra-filtered using collodion membrane and supernatant was obtained which produced brownish black precipitate (H_1) with the addition of HCl. The residue on the membrane, after treatment with 5% HCl at 70°C for 30 min, was again extracted with hot 0.5% NaOH, and a brownish black precipitate (H_2) was obtained. In addition, each mycelium of *S. viridochrom* and *Botrytis cinerea* was placed in a petri dish, and submerged and air-dried repeatedly 20 times at 30°C, then extracted with hot alkali to obtain HA (H_3) by the above procedure.

As shown in Fig. 8-4 and Table 8-3, the absorption spectra of HAs extracted from the same materials were very similar in shape, though the *RF* value was larger in the order: $H_1 < H_2 < H_3$. These HAs were not, however, completely identical in shape of their absorption spectra to soil HAs, but were more or less analogous.

Judging from the $(K_0 - K_{14})/K_0$ value, these HAs were fairly stable and comparable to soil HAs, but their B/A values were rather high, indicating

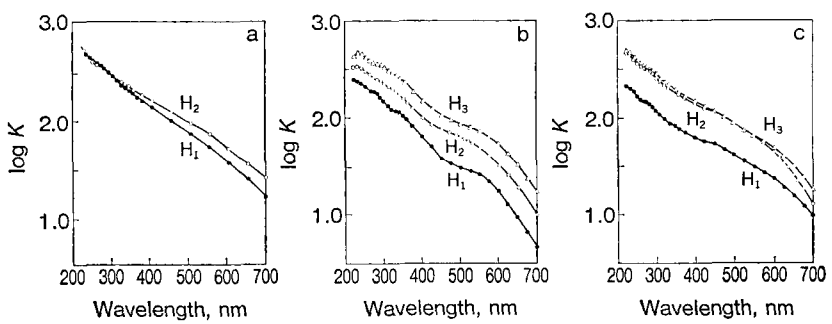


Fig. 8-4. Microorganism pigment humic acids. a: *Streptomyces pheochromogenes*; b: *S. viridochrom*; c: *Botrytis cinerea*.

TABLE 8-3
Properties of microorganism pigment humic acids.

Sample		*RF*	$\Delta \log K$	$(K_0-K_{14})/K_0$ (%)	*B*/*A* (%)
Streptomyces pheochromogenes	H_1	22	0.568	12.0	43.9
	H_2	30	0.462	5.2	57.1
S. viridochrom	H_1	11	0.532	14.7	63.5
	H_2	20	0.481	9.1	44.0
	H_3	33	0.438	—	60.8
Botrytis cinerea	H_1	14	0.503	30.4	59.3
	H_2	27	0.450	27.3	61.3
	H_3	30	0.511	—	75.0

that they were contaminated by abundant colorless organic matter, readily decomposable by alkaline permanganate solution. When they were oxidized by permanganate in alkaline solution, white and flocculent precipitate was formed.

It is evident that these microorganism pigment HAs are not identical to soil HAs, however, they are comparatively stable and their absorption spectra are fairly similar. Therefore, it may be possible that they can transform and become closer to soil HAs under the influence of microbial and chemical action in soils. They may, thus, be called forerunners of soil HAs, although experimental verification is necessary, especially to determine of which type of HAs.

Artificial humic acids prepared from ISL pigment and other materials by treatment with concentrated sulfuric acid

In the course of a series of studies on artificial HAs, it was presumed that soil HAs, especially A type, must contain not only benzene rings but also condensed rings as part of their structural unit. Therefore, the author attempted to prepare an artificial HA by treatment with concentrated sulfuric acid from the reddish brown pigment which was produced by *Penicillium islandicum* spp. and contained various hydroxyanthraquinones. A number of soil HAs and organic compounds were subsequently treated with sulfuric acid and changes in absorption spectrum and stability were examined (Kumada, 1958).

The reddish brown pigment of *P. islandicum* (ISL pigment) was placed in a Kjehldahl flask and conc. H_2SO_4 added. After gently warming to dissolve the pigment, the deep red solution was boiled under suction. The solution darkened rapidly with the elevation of temperature, then became blackish violet in color and very viscous. With further boiling, the solution changed

gradually from blackish brown, through dark reddish brown, to deep red, and at the same time the viscosity decreased. A small portion of the solution was taken out several times in the course of boiling and poured in a large volume of water to produce blackish brown precipitate. The precipitate was transferred to a filter paper, washed with water, then dissolved in dilute alkali. The precipitate (ISL HA) obtained by acidifying the alkaline solution was filtered, washed with water, and dissolved by adding 0.1% NaOH. This alkaline solution was used immediately for determining absorption spectrum and stability. The results are given in Fig. 8-5 and Table 8-4.

The ISL pigment has three distinct absorption bands at about 600, 550, and 400 nm, but these bands vanished after 30 min boiling, and the absorption at the visible and UV region increased remarkably (see No. 27 in Fig. 8-5). The absorption curve became nearly straight in shape, and its inclination towards the axis of wavelength smaller. With further boiling, the solution de-

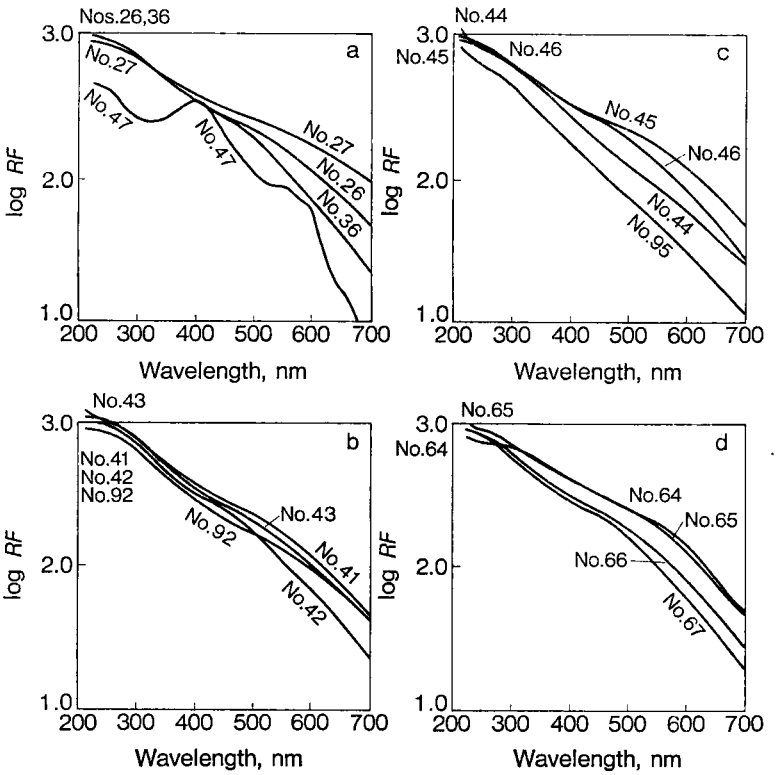


Fig. 8-5. Changes in absorption spectra of ISL pigment, soil humic acids, and glucose by treating with conc. H_2SO_4. a: ISL pigment; b: Tanemori HA (A type); c: Kosudo HA (B type); d: glucose.

TABLE 8-4
Optical properties and stability of humic acids treated with conc. H_2SO_4.

Material	No.	Duration of boiling	*RF*	$\Delta \log K$	*B*/*A* (%)	$(K_0-K_{14})/K_0$ (%)
ISL pigment	47	0	163	0.756	70.5	97.2
	27	30 min	478	0.318	54.1	30.7
	26	1 hr	301	0.472	51.5	29.2
	36	2	184	0.675	49.5	18.0
Tanemori	92	0	254	0.501	35.8	10.5
(A type)	41	15 min	301	0.470	39.9	24.7
	43	25	264	0.537	42.8	19.1
	42	45	—	0.675	—	18.1
Kosudo	95	0	47	0.780	53.8	24.5
(B type)	44	30 sec	76	0.678	60.0	33.6
	45	5 min	189	0.477	52.9	34.3
	46	15	131	0.612	54.0	31.8
Glucose	64	5 min	364	0.502	59.8	27.9
	65	15	376	0.460	60.1	40.5
	66	45	225	0.569	55.2	31.3
	67	1.5 hr	169	0.685	56.1	17.3

colorized gradually and the absorption curve of the HA became steeper (see Nos. 26 and 36 in Fig. 8-5). It must be noted that the absorption spectrum of No. 26 is very similar in shape to A type HA, which was also ascertained from the IR spectrum.

The same experiment was conducted on soil HAs and various organic compounds such as anthraquinone, hydroquinone, glucose, lignin, terramycine, catechol, and naphthazarin. All of these materials dissolved completely in conc. H_2SO_4 giving a deep red or yellow solution, and the change in color in the boiling process was nearly the same as was the case of the ISL pigment. A closer examination of their absorption spectra, however, disclosed that more or less obvious differences could be recognized between them presumably depending on the kind of materials (Fig. 8-5).

Data in Table 8-4 show that the ISL pigment was originally very unstable, but the stability of the ISL HAs increased greatly and became comparable to that of soil HAs, though somewhat lower. Stability of the other artificial HAs was more or less lower than that of the ISL HAs. It may be said that the ISL HAs, especially No. 26 serve as the model for A type HA.

Furthermore, as mentioned, the changes in shape of absorption spectra of the materials in the boiling process were approximately analogous, suggesting that the reaction (wet combustion) processes occurring here were essentially the same.

The course of treatment of organic materials with boiling sulfuric acid may give rise to various reactions, *e.g.*, aromatization, oxidation, dehydration, condensation, sulfonation; the reaction process may be regarded as an initial stage of carbonization, followed by decomposition to form CO_2 and H_2O. At the same time, humification can be regarded as an initial stage of carbonization too, because A type HA has turbostratic structure. Then, both processes are in a sense analogous to each other.

Comments

(1) Hydroquinone HA was quite analogous in shape of absorption spectrum to that of A type HA, but its stability was distinctly lower. The hydroquinone HA had a turbostratic structure which was less developed than that of the A type (see Chapter 10). These findings suggest strongly that the "humification" process of hydroquinone under laboratory or soil conditions can serve as a model of the formation of A type.

(2) Artificial HAs obtained from various ranks of coal by treatment with $KClO_3$ and HNO_3 corresponded to A, B or Rp type HAs, suggesting the structural similarity between the two groups. The fact that anthracite HA was similar to A type with respect to absorption spectrum and stability was especially noted.

(3) The change in absorption spectrum of lignin by treatment with HCl suggests that lignin can serve as a source of Rp and B type HAs. Milder preparative conditions should be pursued for the models of soil HAs.

(4) There is a possibility that melanin- and microbial pigment HAs can be transformed into soil HAs, and this transformation under soil conditions should be investigated.

8.2. Formation of Hydroquinone Humic Acid as Affected by Aluminum and Iron

As discussed in Chapter 6, there exist at least two kinds of soils in which A type HA is predominant: Black soils of Japan originated from volcanic ash and calcareous soils including various great soil groups. It may be reasonable to suppose that the two kinds of soils have their own conditions for the formation of A type, although it was demonstrated that the A type HAs obtained from them have a turbostratic structure in common (see Chapter 10).

Hydroquinone- and catechol HAs formed by autoxidative polymerization in alkaline solution showed the existence of a turbostratic structure (Pollack *et al.*, 1971; Matsui *et al.*, 1984) and can therefore be regarded as models for the A type of calcareous soils.

In Black soils, it is suspected that amorphous oxides of aluminum and

iron, allophane and imogolite might be related to the formation of A type under acid soil conditions, because the Black soils are characterized by the predominance of these inorganic constituents. To verify this supposition, a series of model experiments using hydroquinone, catechol and pyrogallol as starting materials was carried out (Kumada and Kato, 1970; Kumada and Ohta, 1971; Kondo, 1978). An example of the experiments is described below.

To 660 mg hydroquinone alone or with various amounts of $AlCl_3$ or $FeCl_3$ put in a 50 ml beaker, 20 ml of 0.1N Na-acetate (pH 6.5, 5.5, 4.5 or 3.5) was added. The beakers were covered with aluminum foil and placed at 30°C. After 14 days, the solution was acidified with H_2SO_4, and the precipitate (HA) was transferred to a filter paper, and successively washed with dil. H_2SO_4 and H_2O. The HA was dissolved in 0.01N NaOH, and analyzed by the Nagoya method of humus composition analysis. In addition, 660 mg of hydroquinone was dissolved in 20 ml of 0.01N NaOH, and the HA fraction was analyzed similarly 7 days after dissolution.

The absorption spectra of hydroquinone HAs are illustrated in Fig. 8-6.

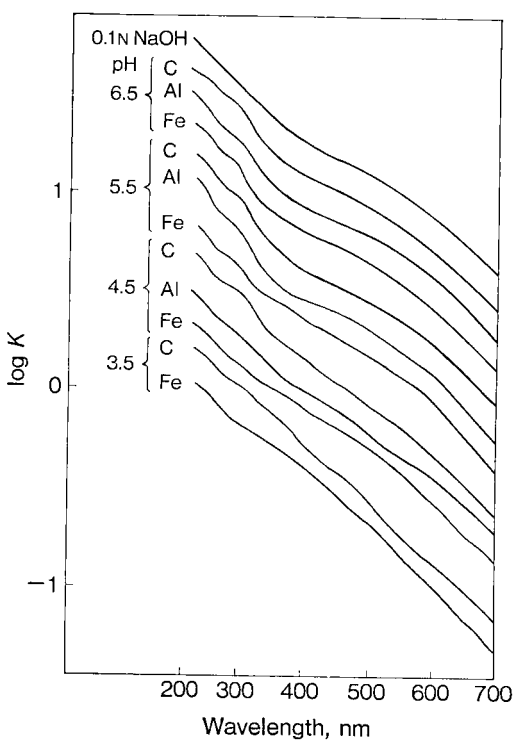


Fig. 8-6. Absorption spectra of hydroquinone humic acids. C: control; Al: 10^{-3}M $AlCl_3$: Fe: 10^{-3}M $FeCl_3$.

The shapes of absorption curves were mostly similar to each other and to that of A type HA, except that most of them had a shoulder-like absorption in the UV region. The analytical results listed in Table 8-5 show the following: (i) In the acid solutions, the yield of HA increased with increasing pH. (ii) At the same pH value and the same concentration of Al and Fe, the yield was in the order: control plot < Al plot < Fe plot. (iii) The yields in the Fe and Al plots increased with their increasing amounts, respectively. (iv) The yield of HA in the alkaline solution was remarkably high. (v) The *RF* values of HAs were nearly comparable to that of A type HA, but the $\Delta \log K$ values were in most cases lower.

TABLE 8-5
Yields of hydroquinone humic acids and their *RF* and $\Delta \log K$ values.

Plot	pH	Amount of additive (M)	Yield[a]	*RF*	$\Delta \log K$
Control	6.5		11.7	124	0.387
	5.5		7.40	120	0.378
	4.5		3.64	92	0.491
	3.5		2.90	82	0.622
Al[b]		10^{-3}	21.3	122	0.350
	6.5	5×10^{-3}	21.8	128	0.349
		10^{-2}	19.5	129	0.364
		10^{-3}	9.64	113	0.415
	5.5	5×10^{-3}	10.5	119	0.381
		10^{-2}	11.4	122	0.379
		10^{-3}	2.58	139	0.418
	4.5	5×10^{-3}	2.76	131	0.430
		10^{-2}	2.90	143	0.427
Fe[b]		10^{-4}	30.8	127	0.340
	6.5	5×10^{-4}	40.0	127	0.373
		10^{-3}	43.0	117	0.377
		10^{-4}	19.7	131	0.363
	5.5	5×10^{-4}	25.5	132	0.407
		10^{-3}	31.5	122	0.421
		10^{-4}	12.4	116	0.492
	4.5	5×10^{-4}	18.0	129	0.490
		10^{-3}	22.5	134	0.444
		10^{-4}	7.24	93	0.501
	3.5	5×10^{-4}	9.36	122	0.545
		10^{-3}	12.2	116	0.566
Alkali	0.1N NaOH		139	124	0.395

[a] Expressed as ml of 0.1N $KMnO_4$ consumed.
[b] Al: $AlCl_3$; Fe: $FeCl_3$.

It may thus be concluded that Al and especially Fe salts accelerate the formation of hydroquinone HA in acid conditions. The yield of HA in 0.1N NaOH was far larger than that in acid conditions. In the case of Black soils, however, active alumina abundantly present is considered to combine with HA to form stable Al-humate and contribute to its accumulation.

In the model experiments conducted by the author and associates, Mn oxides were not taken into consideration. However, a series of model experiments carried out by Shindo (Shindo and Huang, 1982, 1984a,b, 1985a,b; Shindo and Higashi, 1986) showed that Mn(IV) oxide and Mn-bearing silicates, especially tephroite, accelerate remarkably the oxidative polycondensation of polyphenols. The accelerating effect of Mn compound was confirmed by an experiment in which the yield of hydroquinone HAs in 0.1N Na-acetate solution (pH 6.0) containing 10^{-3}M $FeCl_3$ or $MnCl_2$ at 30°C during a 1- to 4-week incubation period was 2.6 to 3.1 and 7.4 to 8.6 times larger than those of the control plot (Kumada and Nishio, unpublished data). It is added that allophane and imogolite deferrated by the Mehra and Jackson method (1965) did not show the accelerating effect.

The above model experiments are incomplete and many problems remain to be solved. But the findings obtained to date seem to indicate that this is an effective means to understand various phenomena occurring in soil, and that methodology for model experiments should be established.

Chapter 9

Chemical Properties of Various Types of Humic Acid

HAs are divided into several types based on their optical properties. From the standpoint of humus chemistry, the chemical properties of these HA types are a most important factor. In Chapter 5 it was shown that the elementary composition of various HA types can be discriminated from each other. Some of the chemical properties are dealt with in this chapter. It is to be regretted that the items of chemical properties which have been studied in connection with the types of HA and the degree of humification are rather limited, and the results are still fragmentary.

9.1. Oxygen-containing Functional Groups

Levels of total acidity and carboxyl, phenolic and alcoholic hydroxyl, carbonyl, and methoxyl groups of various types of HA were determined by Kumada and Kawamura (1968a) and Tsutsuki and Kuwatsuka (1978a). Suzuki and Kumada (1972) determined the contents of the above functional groups except for the carbonyl group for Rp(2) type HAs. The analytical data reported by these workers are all plotted in Fig. 9-1.

Rather wide ranges of the values for each analytical item may be partly due to the fact that different methods were used for a single item. For instance, larger values of total acidity and carbonyl group for respectiev types of HAs were reported by Tsutsuki and Kuwatsuka than by Kumada and Kawamura. For their determinations, the former used the methods of Martin *et al.* (1963) and Schnitzer and Riffaldi (1972), while the latter used the methods of Brooks and Sternnel (1957) and Fritz *et al.* (1959). On the other hand, the carboxyl and total hydroxyl groups were respectively determined according to the methods of Blom *et al.* (1957) and DeWalt and Glenn (1952) by

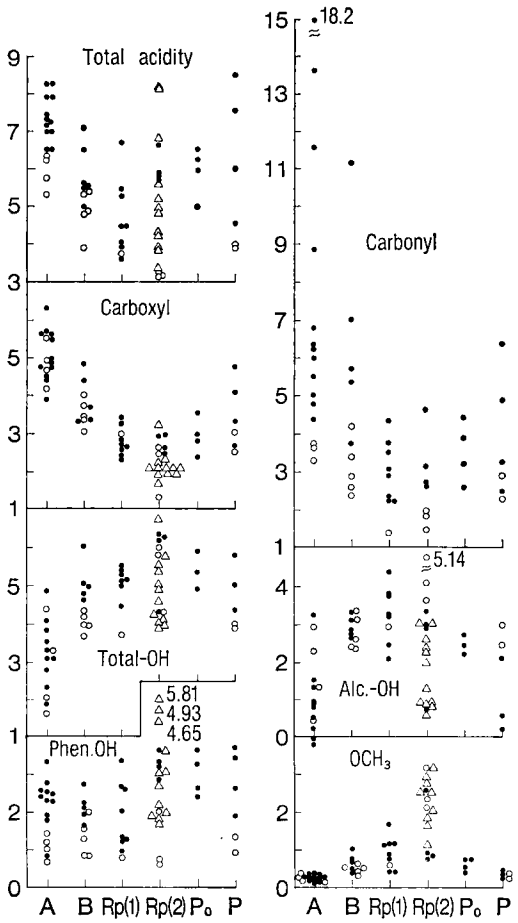


Fig. 9-1. Oxygen-containing functional groups (me/g) of humic acids. ○: Kumada and Kawamura (1968a); ●: Tsutuki and Kuwatsuka (1978a); △: Suzuki and Kumada (1972).

both researchers. For the determination of methoxyl group, Tsutsuki and Kuwatsuka used the method of Vieböck and Brecher (1930), and Kumada and Kawamura used the Zeisel method; no difference due to the different methods was observed.

Phenolic hydroxyl content calculated by subtracting the carboxyl content from the total acidity, and alcoholic hydroxyl content by subtracting the phenolic hydroxyl content from the total hydroxyl content would be affected by the methods used for the direct determination of functional groups, especially total acidity. Functional groups of Rp(2) type HAs reported by

Suzuki and Kumada were determined by the methods used by Kumada and Kawamura. Tsutsuki and Kuwatsuka (1978a) remarked on many problems involved with the determination of functional groups, especially phenolic and alcoholic hydroxyl.

Regarding the data in Fig. 9-1: (i) The levels of the total acidity and carboxyl and carbonyl groups tended to increase in the order of Rp(2) type $<$Rp(1) and P_0 type$<$B type$<$A type, and the reverse was true for the total hydroxyl and methoxyl groups. (ii) In the phenolic and alcoholic hydroxyl groups, there were no tendencies among various types of HA, except that both the hydroxyl contents of Rp(2) type HAs showed wider ranges than those of other types, and the alcoholic hydroxyl contents of A type HAs were smaller than others. This may be partly due to the inaccuracy of these values for the reasons stated by Tsutsuki and Kuwatsuka (1978a). (iii) As for P type HAs, the levels of all functional groups except for the methoxyl group ranged widely, and it was evident that with the increase of the Pg fraction in P type HAs, the amounts of total acidity, carboxyl, phenolic hydroxyl, and carbonyl groups increased and those of alcoholic hydroxyl and methoxyl groups decreased.

Using the HA samples (except for P type) plotted in Fig. 9-1, Tsutsuki and Kuwatsuka (1978a) conducted linear and logarithmic regression analyses of the atomic ratios of the oxygen of O-containing functional groups to total-C on *RF* values, and obtained these correlation coefficients (γ): 0.844*** (logarithmic) for O_{COOH}/C, 0.799*** (logarithmic) for $O_{C=O}/C$, 0.234 (linear, N. S.) for $O_{phen.\,OH}/C$, -0.815*** (linear) for $O_{alc.\,OH}/C$, and -0.768*** (linear) for O_{CH_3O}/C. They further stated that, when a few exceptions were excluded, the carbonyl and carboxyl contents showed a highly significant linear association (γ: 0.913***), and that the ratios of oxygen contents accounted for by the sum of carboxyl and carbonyl groups to total oxygen contents of HAs increased with *RF* values, and amounted to about 80% for A type HAs.

These results suggest that carboxyl and carbonyl groups are important structural components, and the formation of these functional groups proceeds in parallel in the process of humification.

It is added that Arai and Kumada (1977a) proposed a conductometric titration method which enables fractionation of the functional groups of HAs into three clusters of different dissociation constants, designated as S (strong), W (weak) and VW (very weak) clusters. They showed that the sums of the functional groups and the ratios of the S cluster to the sums likewise increased in the order of Rp type$<$B and P types$<$A type (Arai and Kumada, 1977b). The total acidities determined by conductometry were nearly equal to the sums of carboxyl determined by Blom's method and phenolic

hydroxyl determined by Folin's method as modified by Kuwatsuka and Shindo (1973).

9.2. Nitrogen Distribution in Humic Acids

Tsutsuki and Kuwatsuka (1978b) determined the levels of amino acid, ammonia, amino sugar, hydrolyzable-N, non-hydrolyzable-N, and total-N in HAs and discussed their relationships to the degree of humification (*i.e.*, *RF* values) or the types of HA. Part of their results are described below.

Amino acid composition of humic acids

Amino acid compositions in the 6N HCl hydrolysates of 4 HA samples are shown in Table 9-1. Each molar amount of Asp, Glu, Gly, and Ala was about 10% or more of the total amount of acids. The composition of these 4 amino acids was very similar among the 4 HAs though they were different

TABLE 9-1
Relative molar distribution of amino acids in humic acids (%) (Tsutsuki and Kuwatsuka, 1978b)

Name *RF* Type	Humic acid Tenmondai 144 A	 Kuragari 61 B	 Hichiso 51 B	 Kisokoma(F) 20 Rp(2)	Average	Standard deviation
Acidic						
Aspartic acid	14.6	14.3	13.4	13.3	13.9	0.64
Glutamic acid	11.5	9.59	10.6	10.5	10.5	0.78
Neutral (non-polar)						
Glycine	12.6	12.8	11.6	12.3	12.4	0.51
Alanine	11.9	10.6	10.9	10.4	10.9	0.65
Valine	7.76	8.36	5.86	7.63	7.40	1.08
Isoleucine	4.13	4.16	4.66	4.86	4.45	0.37
Leucine	5.59	6.32	7.14	7.87	6.73	0.99
Phenylalanine	3.45	3.76	3.87	4.08	3.79	0.26
Proline	4.87	6.28	6.51	5.80	5.87	0.73
Neutral (polar)						
Serine	5.28	5.81	6.09	5.54	5.68	0.35
Threonine	6.60	5.36	7.18	5.57	6.18	0.86
Methionine	0.00	0.71	1.14	0.81	0.67	0.48
Tyrosine	0.89	1.40	1.77	2.17	1.56	0.55
Cystine	0.00	0.00	0.00	0.00	0.00	0.00
Basic						
Lysine	5.89	5.57	3.84	4.16	4.87	1.02
Histidine	1.67	1.55	2.66	2.68	2.14	0.61
Arginine	3.31	3.48	2.82	2.27	2.97	0.54

in *RF* values and origin. Each molar amount of Val, Ile, Leu, Phe, Pro, Ser, Thr, and Lys ranged from 4 to 8%, and that of Met, Tyr, His, and Arg ranged from trace to 4% of the total amino acids. These amino acid compositions were similar to those of HA obtained from a Chernozem soil by Khan and Sowden (1971, 1972) and from highly organic tropical volcanic soils by Sowden *et al.* (1976). These results suggest that the protein structure of HAs is unaffected by the degree of humification and the origin of HAs.

Total-N content

Total-N contents were plotted against *RF* values (Fig. 9-2). The plots were grouped together according to types, *i.e.*, A, B, Rp(1), Rp(2), and P_0 type. Total-N contents increased from Rp(2) type to Rp(1) type, and then decreased among Rp(1), P_0, B, and A type with increasing *RF* values. A very significant negative linear correlation (γ: -0.715***) was found between total-N content and *RF* value among the latter 4 types of HAs. As pointed out by Kumada (1955c), the process of HA formation could be divided into two stages according to the change in total-N content. In the first stage, corresponding to the change from Rp(2) type to Rp(1) type, HAs with low nitrogen content are transformed to those rich in nitrogen, because easily utilizable materials such as polysaccharides may be exhausted by soil microbes, leaving behind humified substances or dead biomass richer in nitrogen. The color of HAs, *i.e.*, the degree of humification does not increase during this stage. In the next stage, nitrogen is released again with the increase in the brown color of HA.

Amino acid-N content by 8N H_2SO_4 hydrolysis

The total amino acid-N content in the hydrolysate obtained by treat-

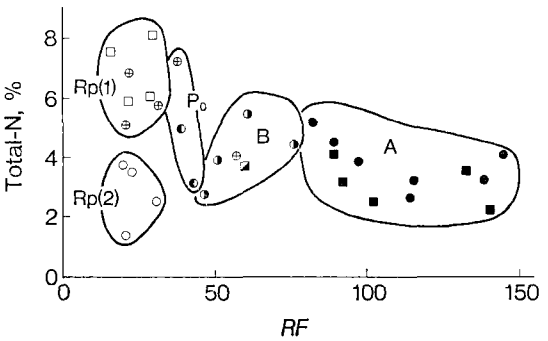


Fig. 9-2. Relationship between total N% and *RF* values of humic acids (Tsutsuki and Kuwatsuka, 1979b)

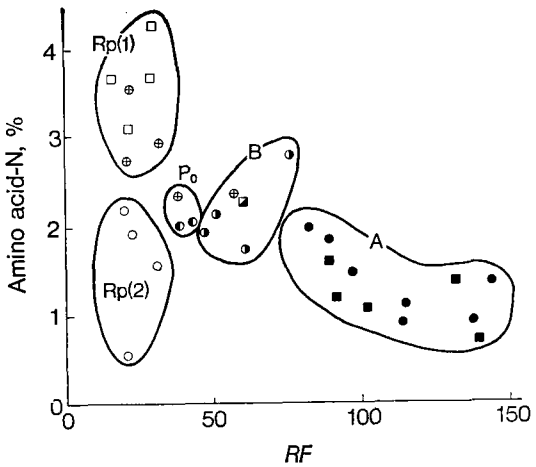


Fig. 9-3. Relationship between amino acid-N% and *RF* value of humic acids. (Tsutsuki and Kuwatsuka, 1978b)

ment with 8N H_2SO_4 at 110°C for 20 hr was determined by TNBS method (a colorimetry using sodium trinitrobenzene sulfonate) according to Satake and Okuyama (1958). The amino acid-N content increased from Rp(2) type to Rp(1) type, and then decreased among Rp(1), P_0, B, and A types with increasing *RF* value (Fig. 9-3); a very significant negative linear correlation (γ: -0.861^{***}) was found between the amino acid-N content and *RF* value among the latter 4 types of HAs.

It should be noticed that various types of HAs are discriminated from each other with respect to nitrogen distribution with transitional changes observed in the order of Rp(2), Rp(1), P_0, B, and A type HAs.

9.3. Amino Acid, Phenol, and Sugar Composition of Acid-hydrolysate

Following the investigation described above, Tsutsuki and Kuwatsuka (1979a) determined the levels of amino acids, phenolic substances, sugars, and total hydrolyzable organic matter of 37 HAs after acid-hydrolysis.

Amino acid content

Total yields of amino acid by 8N H_2SO_4 hydrolysis of HAs ranged from 400 to 2,800 μmol/g, and increased from Rp(2) type to Rp(1) type, then decreased in the order of Rp(1), P_0, B, and A type HAs with increasing degree of humification. Amino acid-carbon is supposed to be the main fraction of the acid-hydrolyzable-carbon in HAs.

Phenolic substance content

The contents of phenolic substances in 8N H_2SO_4 hydrolysates were determined colorimetrically with Folin's reagent, using phenol (C_6H_5OH) as the standard material. Very significant positive linear correlations were found between the yields of total phenolic substances and total amino acids by 8N H_2SO_4 hydrolysis (γ: 0.883***) and between the yields of the former and acid-hydrolyzable organic matter (γ: 0.967***). Here, acid-hydrolyzable organic matter refers to the content of total organic carbon in the supernatant of hydrolysate of HA treated with 8N H_2SO_4 at 110°C for 20 hr, and determined according to Tatsukawa's method (1966).

Similar to the trends observed for amino acids, the total yields of hydrolyzable phenolic substances increased from Rp(2) type to Rp(1) type HAs, and then decreased in the order of Rp(1), P_0, B, and A type HAs with increasing *RF* values (Fig. 9-4). Because of their similar trends, the proteinous substances and hydrolyzable phenolic substances in HAs are presumed to combine with each other and to have similar stabilities, though proof is still needed of their combination. It is also presumed that the combination of the two substances takes place during the first stage of humification (from Rp(2) type to Rp(1) type), and the degradation takes place during the next stage (among Rp(1), P_0, B, and A type HAs).

As seen in Fig. 9-4, the yields of acid-hydrolyzable phenolic substances from Rp(2) type HAs were lower than those from Rp(1) type. The yield

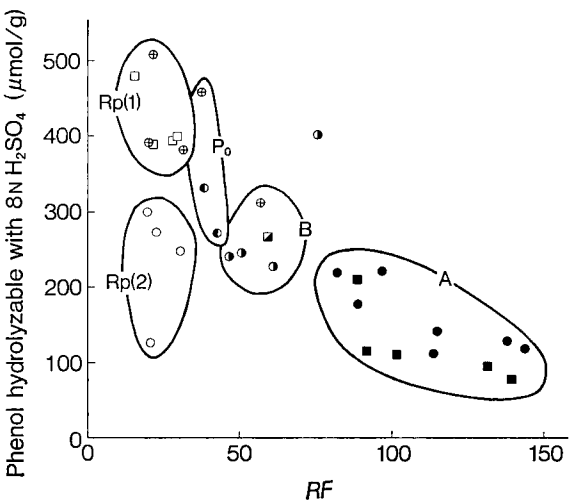


Fig. 9-4. Relationship between the level of acid-hydrolyzable phenol and *RF* values of humic acids (Tsutsuki and Kuwatsuka, 1979a).

was the lowest for the HA (No. 37 in Table 5-1) which was isolated from rotted wood of *Myrica rubra* containing high levels of flavonol (Myricitrin). Lignin is also stable and little degraded by acid-hydrolysis. Therefore, it is presumed that the phenolic substances detected in the acid-hydrolysates of HAs are mainly due to phenolic substances other than lignin and flavonoid. These phenolic substances may be due to hydrolyzable tannins, microbial metabolites and chemical degradation products of residual constituents of plants.

Hexose and uronic acid contents

The contents of hexose and uronic acid in 6N H_2SO_4 hydrolysate (100°C, 2 hr) were determined by the methods of Atake and Seno (1968) and Bitter and Nuir (1962), respectively. The levels of hexose in HAs ranged from 0.5 to 5%, and tended to decrease with increasing *RF* values (Fig. 9-5). A significant negative linear correlation (γ: -0.472*) was found between the hexose level and *RF* value of the HAs. Compared with other HAs of similar *RF* values, the HAs extracted from Rendzina-like soils contained lower levels of hexose. A negative linear correlation between the hexose content and *RF* value was found to be much more significant for the HAs obtained from Rendzina-like soils (γ: -0.842***) than the HAs overall (γ: -0.472*).

The levels of uronic acid ranged from 0.5 to 2.0%. Though no significant linear correlation (γ: -0.319 N. S.) was found between the uronic acid level

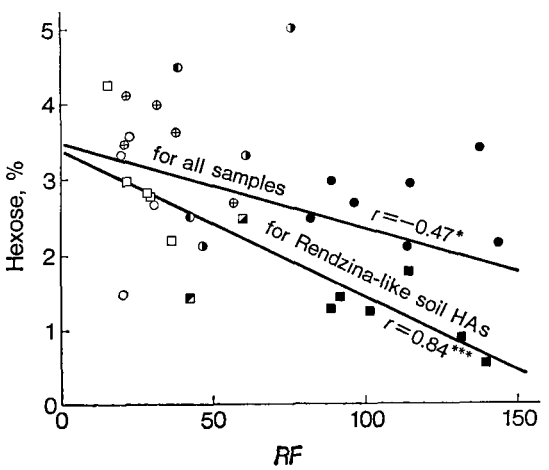


Fig. 9-5. Relationship between the level of hexose and *RF* value of humic acids (Tsutsuki and Kuwatsuka, 1979a). Rendzina-like soil humic acids are denoted by ■, ◪, ◩, and □ for A, B, P_0, and Rp type, respectively.

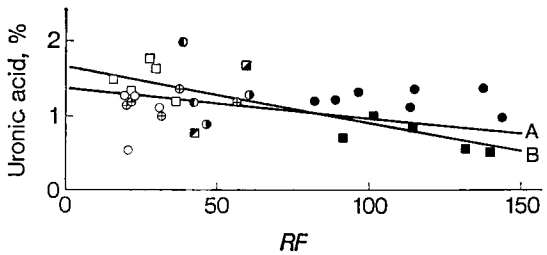


Fig. 9-6. Relationship between the level of uronic acid and *RF* value of humic acids (Tsutsuki and Kuwatsuka, 1979a). A: for all samples (r: $-0.32^{N.S.}$); B: for Rendzina-like soil humic acids (r: -0.78^{**}), which are denoted by the same symbols as in Fig. 9-5.

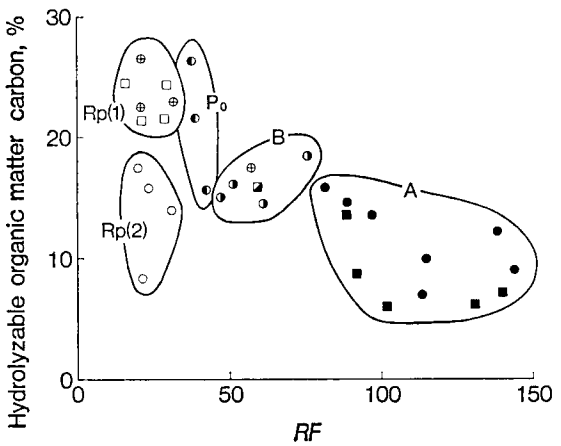


Fig. 9-7. Relationship between the level of hydrolyzable organic matter carbon and *RF* value of humic acids (Tsutsuki and Kuwatsuka, 1979a).

and *RF* value in any samples, a significant negative linear correlation (r: -0.780^{**}) was found between these values for Rendzina-like soil HAs (Fig. 9-6).

Contribution of the carbon content distributed in amino acids, phenolic substances, and sugars as acid-hydrolyzable constituents to the total carbon content of humic acid

Of the total carbon content in HAs, that in amino acids accounted for 4–26%, in hydrolyzable phenolic substances 1–6.7%, in hexose 0.6–3.5%, and in uronic acid 0.3–1.4%. The sum of the carbon contents of acid-hydrolyzable constituents, (A+P+H+U) value, accounted for 5.9–35% of the total carbon. On the other hand, the levels of carbon contained in the supernatant solutions of the 8N H_2SO_4 hydrolysates of HAs, which were deter-

mined directly by Tatsukawa's method, accounted for 5.8–27% of the total carbon. The values of (A+P+H+U) and hydrolyzable organic matter were similar in most of the HAs exmained here, and a very significant positive linear correlation (γ: 0.931***) was found between them. Therefore, a great part of the acid-hydrolyzable fraction of HAs is assumed to be accounted for by amino acids, hydrolyzable phenolic substances, and sugars.

As shown in Fig. 9-7, the pattern showing relationship between the level of acid hydrolyzable organic matter and *RF* value of HAs was similar to that of acid-hydrolyzable phenol (Fig. 9-4).

9.4. Fractionation Experiment

It was stated earlier that an HA is considered to be an assembly of fractions continuously differing in their *RF* and $\Delta\log K$ values. This idea can be demonstrated by dividing the HA into several fractions according to appropriate means, *e.g.*, gel chromatography, and determining the *RF* and $\Delta\log K$ values of each fraction. Here, experimental results supporting the idea are described.

Fractional precipitation

The fractional precipitation technique using alcohol as precipitant (Soc. High Polymer Chem., 1961) was utilized (Kyuma, 1964; Kumada and Kawamura, 1968; Shiroya and Kumada, 1973).

Fractionation procedure

To a solution containing about 150 mg of HA dissolved in 20 ml of 0.2 N NaOH, absolute ethanol was added to a concentration of 20%. After standing overnight, the precipitate was separated by centrifugation. It was then re-dissolved in 0.2N NaOH, alcohol added to the same concentration as above, and the precipitate recovered by centrifugation. Alcohol was added to the combined supernatant solutions to a concentration of 30%; again, the precipitate was recovered as described above. The procedure was continued until the concentration of alcohol became 80% and 7 fractionated precipitates were obtained. 0.2N NaOH can be replaced by 0.2N Na-acetate (pH 7.0) in this procedure (Ohtsuka, 1974c). Each precipitate was dissolved in 0.1N NaOH, precipitated by adding HCl (1:3), transferred to a glass filter, and washed with ether. After drying in a desiccator containing conc. H_2SO_4 for two days, the precipitate was weighed. After the concentration of alcohol was reduced by distillation in a water bath, the 80% alcohol soluble HA fraction was precipitated by adding HCl (1:3) and treated as described above.

TABLE 9-2
Distribution of fractionated humic acids and their optical properties.

	Fraction No.	1	2	3	4	5	6	7	8
	Alcohol conc. (%)	20	30	40	50	60	70	80	80<
Inogashira (A type)	Distribution (%)	0.8	79.0	9.0	0.5	5.4	2.5	2.2	0.4
	RF	261	258	215	163	122	88	72	64
	$\Delta \log K$	0.379	0.434	0.453	0.458	0.606	0.680	0.739	0.675
	HA type	A	A	A	A	A	A	B	B
Kuragari (B type)	Distribution (%)	0.1	0.8	1.0	18.5	36.7	9.9	18.3	15.8
	RF		206	165	138	100	48	33	17
	$\Delta \log K$		0.427	0.488	0.524	0.598	0.810	0.836	0.979
	HA type		A	A	A	A	B	Rp	Rp
Higashi-yama (Rp type)	Distribution (%)	0.2	0.2	0.1	0.5	1.3	81.2	4.9	11.5
	RF				31	37	29	18	14
	$\Delta \log K$				0.721	0.778	0.857	0.965	0.971
	HA type				Rp	Rp	Rp	Rp	Rp

RF and $\Delta \log K$ values of original HAs were 138 and 0.49 for Inogashira, 61 and 0.63 for Kuragari, and 23 and 0.79 for Higashiyama, respectively.

Part of the experimental results are illustrated in Table 9-2. The recovery of acid-precipitated HAs ranged from 82 to 87%. With increasing alcohol concentration, the amount of acid-soluble humic matter also increased, judging from the color of supernatant after centrifugation. This may indicate that some alcohol-soluble material, so-called hymatomelanic acid, remained in the solution, thus lowering the recovery of HA.

The distribution pattern of fractionated HAs varied with the HA samples. When *RF* and $\Delta \log K$ of these fractionated HAs are compared with those of the original HAs given in the table, it is clear that the values of *RF* and $\Delta \log K$ of the fractions showing the greatest precipitation were parallel to those of the original HAs. Also, the greater the degree of humification of the original HA, the smaller is the amount of alcohol required to precipitate the larger part of it.

The distribution pattern of fractionated HAs may be assumed to represent the distribution curve of molecular or particle weights of HA, and the particle weight decreases with the increase of fraction number. The distribution curves are apparently not simple; in some cases, they were very sharp and in other cases very broad. However, the curve may have a bias towards larger particle fractions for A type, medium-size for B type and smaller for Rp type.

It is well established that, in a series of high polymer homologues, the larger particles would be precipitated before the smaller upon the addition of alcohol. However, it is unlikely that all the HAs are composed of the

same homologues, and it cannot be argued that the average particle weight of A type HAs is necessarily larger than that of other types. In this context, the shape and size of HA particles, their acid groups, and other factors should be considered.

A regular relationship between *RF* and $\Delta \log K$ was recognized in the HA fractions, and *RF* values decreased and $\Delta \log K$ values increased with an increase in fraction number. This indicates that an HA is an assembly of fractions with continuously differing *RF* and $\Delta \log K$, and there exists an inverse proportionality between these *RF* and $\Delta \log K$ values.

Molecular size distribution

Although numerous studies have been conducted to determine the molecular weight and molecular size of HAs (Orlov *et al.*, 1975; Swift and Posner, 1972; Stevenson, 1982), several of their restrictive properties such as dissociation of functional groups, polydispersiveness, limited solubility, strong light absorption in the visible range, interaction with gel materials, heterogeneous composition, and lack of standard material still hamper the precise determination of molecular weight. Furthermore, the values of the average HA molecular weight seem to be of less practical importance if no information on the molecular weight distribution is supplied with the data. The relationships between the molecular size distribution of HAs and the degree of humification, their origin, as well as their chemical composition also have not been fully documented. To tackle these problems, various kinds of HAs with different degrees of humification should be analyzed, and a rapid and reproducible chromatographic technique should be developed.

To this end, Tsutsuki and Kuwatsuka (1984) applied the permeation chromatography method of HAs on two types of packing materials: porous silica (μ Bondagel) and porous glass (Controlled Pore Glass, CPG). A part of the experimental results on the molecular size distribution of HAs and its relationship with the degree of humification and other HA properties is given below.

Two HAs, Tenmondai (A type) and Higashiyama (B type), were fractionated on a plastic column (85 cm × 25 mm i.d.) packed with CPG-170. A borate buffer (pH 9.1, ionic strength 0.1) was used as eluent. Eluted HA was detected by a differential refractometer, and the effluent was fractionated into 5 ml fractions by a fraction collector. For each fraction, UV- and visible absorption spectrum, optical densities (O.D.) at 600 and 400 nm, $\Delta \log K$, *RF* and organic carbon content were determined.

Elution curves for Tenmondai and Higashiyama HAs are shown in Figs. 9-8 and 9-9. The Tenmondai curves of O.D. at 600 nm, O.D. at 400 nm, and ΔRI (differential refractive index) had peaks at 95, 100, and 107 ml

of elution volume, respectively. The peak of ΔRI which detects both colored and non-colored materials coincided with that of organic carbon content. The fraction with the highest *RF* value was eluted at 90 ml, which was even earlier than the peak of O.D. at 600 nm. These trends showed that dark-colored substances had larger molecular size than non-colored substances in the permeated fraction. *RF* values of eluted HA fractions were highest in the middle molecular size fractions, and were lower in the excluded fractions which had the largest molecular size. On the other hand, $\Delta\log K$ values were almost identically low among the fractions eluted before the peak of *RF*.

RF value is defined as the optical density at 600 nm of HA per unit concentration, therefore, it reflects not only the degree of humification of a humified component, but also the composition of colored and non-colored fractions in HAs. The value of $\Delta\log K$, on the other hand, represents the inclination of the absorption curve in the visible range, and therefore it is not affected by the coexistence of non-colored materials. A low $\Delta\log K$ value indicates the occurrence of highly humified components in the fraction.

Behavior of $\Delta\log K$ values of eluted fractions showed that highly hum-

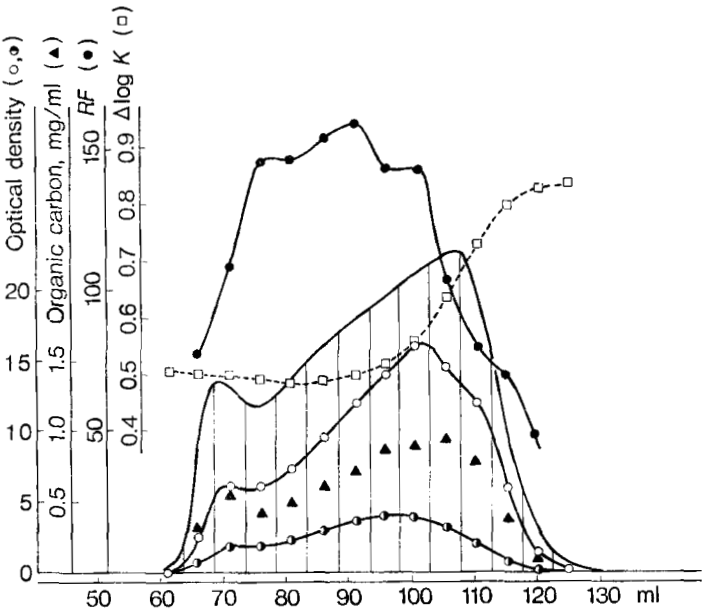


Fig. 9-8. Elution of Tenmondai humic acid (A type) on CPG-170 with a borate buffer (pH 9.1) as monitored by ΔRI, optical densities at 400 and 600 nm, organic carbon, *RF* and $\Delta\log K$ (Tsutsuki and Kuwatsuka, 1984). ●: *RF*; □: $\Delta\log K$; ○: optical density (600 nm); ◑: optical density (600 nm); ——: ΔRI (determined at arbitrary scale); ▲: organic carbon.

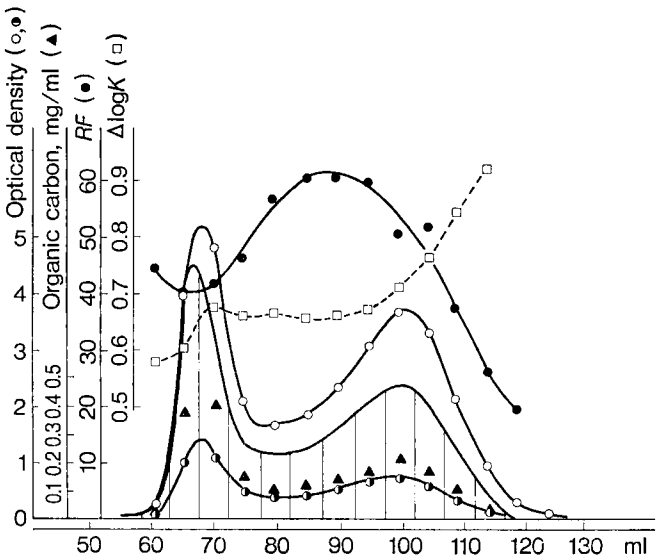


Fig. 9-9. Elution of Higashiyama humic acid (B type) on CPG-170 with a borate buffer (pH 9.1) as monitored by ΔRI, optical densities at 400 and 600 nm, organic carbon, *RF* and $\Delta \log K$ (Tsutsuki and Kuwatsuka, 1984). Notations are the same as in Fig. 9-8.

ified components also existed in the fractions eluted before the peak of *RF* value appeared. Relatively low *RF* values of excluded fractions showed, meanwhile, that non-colored components such as polysaccharide contribute to these large molecular size fractions. Baker *et al.* (1967) also showed that the molecular size of soil polysaccharides was much larger than that of colored materials. After the peak of *RF* value was reached, *RF* values of fractions decreased and $\Delta \log K$ values increased with increasing elution volume.

The elution curve of Higashiyama HA (Fig. 9-9) was characterized by a larger excluded fraction than that of Tenmondai HA. The peak of ΔRI and O.D. at 400 nm was eluted earlier in Higashiyama HA than in Tenmondai HA, which suggests the greater contribution of large molecular size components in the former. The patterns of changes in the *RF* and $\Delta \log K$ values were almost the same as those of Tenmondai HA. The change in these parameters suggests that the middle molecular size fraction had the highest degree of humification, and that highly humified components exist together with the many non-colored components in the excluded fraction. Similar results were obtained with an Rp(1) type HA.

Tsutsuki and Kuwatsuka pointed out on the results obtained by both μ Bondagel and CPG chromatography:

(1) Elution volumes of HAs depended on the ionic strength of eluents.

(2) Average molecular size of A type HAs was smaller than that of B and Rp types. Molecular size distribution of A type was narrower than that of other types.

(3) Elution curves of different HAs were very similar to each other.

These results clearly show that an HA is an assembly of fractions differing in their RF and $\Delta\log K$ values. However, the relationship between RF and $\Delta\log K$ values of HA fractions does not seem as simple as observed in the fractional precipitation experiment.

9.5. Viscosimetric Characteristics

Since HAs are a group of acidic, dark-colored, high molecular weight heteropolycondensates, high molecular chemistry should be an effective approach to elucidate the nature and properties of these substances. For this reason, Kumada and Kawamura (1965) investigated HA viscosimetric properties with special reference to HA type and the intrinsic viscosity and change of reduced viscosity with pH were determined.

Intrinsic viscosity

The HA was dissolved in 0.1N NaOH at concentrations of 1.0, 0.75, 0.5, and 0.25%. Viscosity was measured in a constant temperature bath (25±0.002°C) by Ostwald viscosimeters with flow times of about 300 sec for distilled water at 25°C. Intrinsic viscosity $[\eta]$ was obtained by extrapolating the values of reduced viscosity (η_{sp}/c) to zero concentration, where η_{sp} and c are specific viscosity $(\eta_{rel}-1)$ and concentration, respectively.

The intrinsic viscosity of HA is shown in Fig. 9-10. Because of the small sample number, it was difficult to find any definite regularity between $[\eta]$ and HA type, but the $[\eta]$ values tended to increase in the order of Rp(2) type, Rp(1) type, B type, and then decreased to A type. The values for P type may correspond to those for B type.

Change of reduced viscosity with pH

Free HA solution was prepared by passing sodium humate dissolved in a small volume of 0.1N NaOH through columns of Amberlite IRA-100 OH-form and IR-120 H-form. By adding different amounts of 0.5 N NaOH to each free HA solution, a series of 0.5% HA solutions at different pH values was prepared, and their reduced viscosity was obtained.

The change of reduced viscosity of HA solutions with increasing pH is shown in Fig. 9-11. It can be seen that, with an increase in pH and progressive neutralization of free HA, the viscosity decreased to a minimum

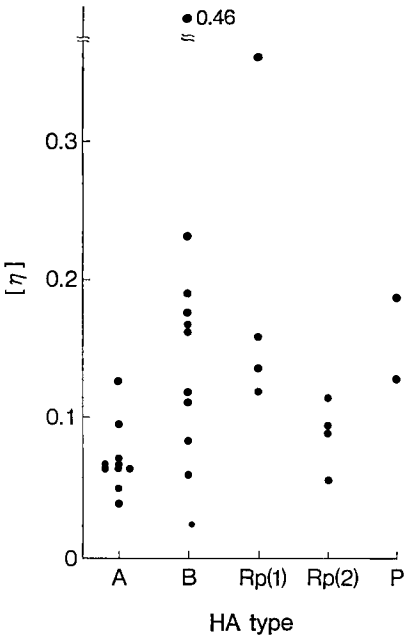


Fig. 9-10. Intrinsic viscosity of various types of humic acid.

and subsequently increased towards neutral and alkaline pH values. Careful examination of those curves reveals that the HAs are divided into four groups. Group I: All the A type HAs belonged to this group. The inclination of descent of the first half curve and the inclination of ascent of the second half curve were the lowest of all groups and the pH value of minimal reduced viscosity was near 5. Group II: B type HAs belonged to this group. The inclination of the first half curve was similar to that of Group I, but the inclination of the second half curve was pronounced. The pH of the minimal point was near 5. Group III: Rp(1) type and P type HAs belonged to this group. The inclination of descent of the first half curve was large, the inclination of ascent of the second half was similar to that of Group II, and the pH of the minimal point was 5 to 5.5. Group IV: The Rp(2) type HAs belonged to this group. The inclination of descent of the first half curve was far larger than that of Group III, the inclination of ascent of the second half was similar to that of Group III, and the pH of the minimal point was 5.5 to 6.

A complete explanation of these η_{sp}/c *vs.* pH curves is not yet possible, but the following explanation may be tentatively acceptable. Free HA solution prepared by passage through columns of ion exchange resins may be

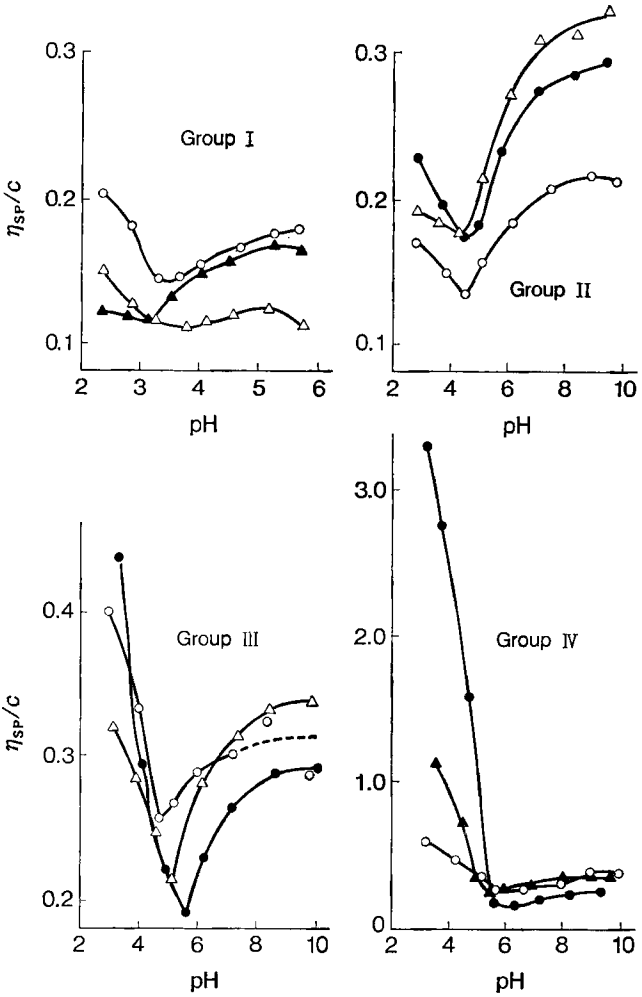


Fig. 9-11. Change of reduced viscosity of humic acids with pH.

assumed to be in a micelle colloid state; the HA particles would more or less associate with each other to form micelles. The reason for this is that the electrostatic repulsion among HA particles would be retarded because of the low degree of dissociation of carboxyl groups. Furthermore, it is presumed that the degree of association increases in the following order: A type<B type<Rp(1) and P types<Rp(2) type, because the ranges of carboxyl group contents for each type(s) were 5.55 to 4.15 me/g, 4.00 to 3.04 me/g, 2.99 to 2.50 me/g, and 2.62 to 1.33 me/g, respectively. According to

the results reported by Arai and Kumada (1977b), the acidic strength of HAs decreased presumably in this order. This assumption is also supported by the pH values of free HA solutions: 2.75 to 2.88 for A type, 2.78 to 3.22 for B type, 2.95 to 3.23 for Rp(1) and P types, and 3.30 to 3.58 for Rp(2) type, indicating that the acidic strength of HAs decreased in this order. It must be noted that Rp(2) type HAs, especially those obtained from farmyard manure, were turbid immediately after passage through ion exchange resins, and the coagulation and precipitation of HA particles were soon observed when allowed to stand. The other HA solutions were very stable, and no flocculation occurred for a long time. Thus, high reduced viscosity for free HA solutions may be explained by micelle formation and this is highest for Rp(2) type HAs.

By adding small amounts of alkali, the association of HA particles would probably be destroyed because of the increase of electrostatic repulsion, and micelle colloids would be transformed into molecular colloids resulting in a decrease of viscosity. The extent of descent of the first half curve seemed to be in the order of A type=B type<Rp(1) and P types< Rp(2) type, in inverse proportion to their magnitude of acidity or the degree of dissociation of acidic groups. When more alkali was added beyond the point of minimal viscosity, the reduced viscosity increased remarkably.

Kagawa (1956) stated that the increase of reduced viscosity with increasing degree of neutralization is a common feature of linear polyelectrolytes of the weak acid type. On the contrary, the reduced viscosity of polyelectrolytes of strong acid type hardly changes with increasing degree of neutralization. This theory may be applicable to HA. The less pronounced elevation of the second half of the curves for A type HAs would indicate that their acidic character is relatively strong compared with other types.

It must be noted that the increase of viscosity that occurs upon neutralization of weak acid-type polyelectrolytes is dependent not only on the shape of the molecule but also on other factors such as molecular weight and charge density. It is accordingly not possible to decide whether the HA molecules are spherical or linear in shape, although it is reasonable to conclude that they are fairly flexible.

According to the data obtained in this investigation, it may be tentatively concluded that the change of reduced viscosity with pH varies with the type of HA. In addition, HAs are thought to be flexible, weak acid-type polyelectrolytes,and the strength of the acid character increases approximately in the order of Rp(2) type<Rp(1) and P types<B type<A type.

SUMMARY

These findings seem sufficient to advocate that the chemical properties of HAs vary regularly with their type and their degree of humification, although data are admittedly still fragmentary.

1. The levels of carboxyl and carbonyl groups tended to increase in the order of Rp(2)<Rp(1) and P_0<B<A type and with the increase of *RF* values, and the reverse was true of the total hydroxyl and methoxyl groups.

2. The levels of total-N, amino acid-N, phenolic substances, and organic carbon in 8 N H_2SO_4 hydrolysate increased from Rp(2) type to Rp(1) type, and then decreased with increasing *RF* values in the order of Rp(1), P_0, B, and A type HAs.

3. The distribution pattern of fractionated HAs by the fractional precipitation technique varied with the type of original HAs. *RF* value of fractionated HAs decreased and $\Delta\log K$ value increased with an increase of fraction number. Similar, though more complicated relationships between *RF* and $\Delta\log K$ values were also observed by permeation chromatography.

4. Change of reduced viscosity with pH varied with the type of HA in the order of Rp(2) type, Rp(1) and P types, B type, A type, and the strength of the acid character increased in this order.

Chapter 10

The Nature and Genesis of Humic Acid

Information on the nature of various HA types is extremely limited, but X-ray diffraction analyses conducted by Matsui *et al.* (1984) confirmed the existence of a turbostratic structure in A type HA, and they succeeded in observing the structure under an electron microscope. Thus, A type HA was considered composed mainly of turbostratic structure and oxygen-containing functional groups. In this connection, the structural models of B and Rp types have been deduced.

In this chapter, the experimental results on the X-ray diffraction analysis of HAs, burning as a possible means of forming soil humus, and environmental evidence on the formation of A type HA are described, and the genesis of various types of HA is discussed.

10.1. X-ray Diffraction

It was mentioned previously that A type HA exhibited a turbostratic structure in X-ray diffraction analysis. Kasatochkin *et al.* (1956, 1964) also proposed a model based on X-ray diffraction analysis composed of condensed aromatic nuclei to which were attached some aliphatic side chains for the HAs extracted from Chernozems. X-ray diffraction studies were also conducted by Simon and Speichermann (1938), Pollack *et al.* (1971), Visser and Mendel (1971), Kodama and Schnitzer (1967) and others, but no systematic understanding of the skeletal structure of HAs and FAs has yet been established.

A few years ago, Matsui *et al.* (1984) obtained normalized X-ray diffraction patterns of HAs, and the (002)-band was further analyzed using the Fourier transformation method.

X-ray diffraction analysis

The diffraction intensity of HA was measured for a powdered sample using a Rigaku Denki X-ray diffractometer and Cu K_α (Mo K_α) radiation in the range sin θ/λ=0.05(0.60) to 0.60(1.08), and was expressed in terms of "reduced intensity I" by the method of Yen *et al.* (1961). That is, the observed intensity, $I_{obs}(\theta)$, was corrected for absorption and polarization factors (Noda and Inagaki, 1964). The corrected intensity, $I'_{obs}(\theta)$, was then normalized to electron units by fitting it to the independent scattering curve in the range sin θ/λ=0.8 to 0.9 (Akamatsu and Takahashi, 1958). Reduced intensity I, was calculated using $I=(A-C)/E$, where A and $C(E)$ are normalized diffraction intensity and incoherent (independent coherent) scatterings, respectively. X-ray diffraction patterns determined are illustrated in Fig. 10-1.

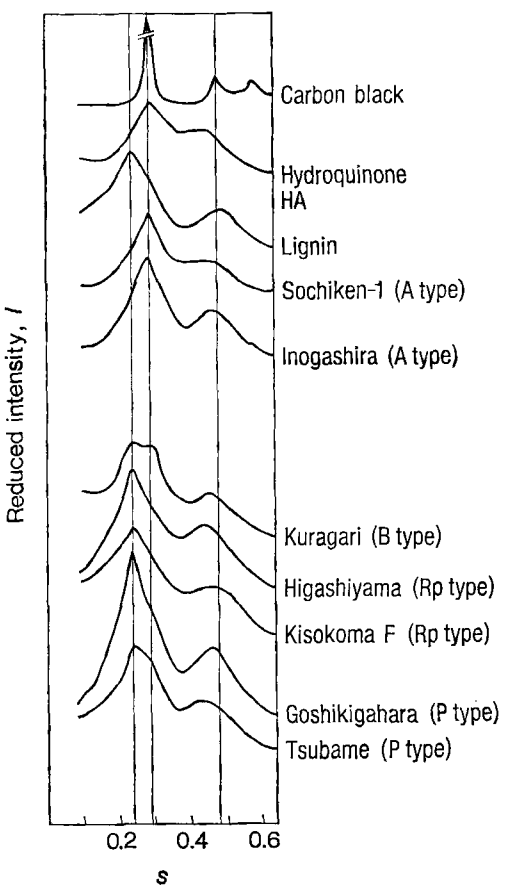


Fig. 10-1. X-ray diffractograms of humic acids and related substances.

Carbon black exhibited sharp peaks at d=3.5, 2.1 and 1.8Å due to (002)-, (10)-, and (004)-bands of graphite, respectively. All A type HAs exhibited two peaks at d=3.5Å and about 2.1Å due respectively to (002)- and (10)-bands of graphite. Rp type HAs exhibited two peaks at d=4.1 and about 2.1Å; the former corresponded to the so-called γ-band. In Kuragari B type HA, there were three peaks at d=4.1, 3.5 and about 2.1Å, and the intensities of the former two were nearly equal. P type HAs containing 4,9-dihydroxyperylene-3,10-quinone derivatives also showed three peaks at d=4.1, 3.5 and about 2.1Å, but the intensity of the peak at d=4.1Å was far stronger than that at d=3.5Å, suggesting that most of the nuclei were not stacked in the manner indicated by the turbostratic structure.

In general, it may be said that soil HAs have (002)- and/or γ-band, and that relative intensities of these bands vary with the types of HAs. The A type HAs showing both (002)- and (10)-bands may be concluded to have turbostratic structure. Rp type HAs have γ-band, and B type HAs γ- and (002)-bands. It is presumed that HAs at the earlier stage of humification (Rp type) have solely a γ-band, and that the (002)-band becomes stronger and the γ-band weaker with the progress of humification, that is, Rp type→B type→A type.

Hydroquinone HA prepared by oxidative polymerization of hydroquinone in 5% NaOH as reported by Kumada and Miyasato (1956), and lignin extracted from rice straw with dioxane had two peaks at d=3.5 and near 2.1Å and d=4.2 and at about 2.1Å, respectively.

Fourier transformation of (002)-band

The Fourier transforms, $P(u)$, defined as the probability of finding a layer at a distance u, for A type HAs and hydroquinone HA are shown in Fig. 10-2. The numbers of peaks, or packing numbers, correspond to the maximum number of layers which are considered to be packed with each other at a distance of interlayer spacing d. The packing numbers of HAs are given in Table 10-1.

In the A type HAs, Sochiken-1 and Inogashira, the amplitude of oscillation was observed to be up to $u \approx 30$Å at an almost constant spacing of 3.5Å, and showed a packing number of 8 to 10. Hydroquinone HA showed a packing number of 5.

The weight ratio of the stacked fraction of n layers ($n \geqq 2$) and $f(n)$, the weight ratio of the nth peak in the transform, are represented by the histograms in Fig. 10-3. For Sochiken-1 HA, this weight ratio was calculated up to seven layers. The main stacked fractions were composed of two (45 wt%) and three (34 wt%) layers. In Inogashira HA, the stacked fractions were also made up of two (60 wt%) and three (29 wt%) layers. Hydroquinone HA con-

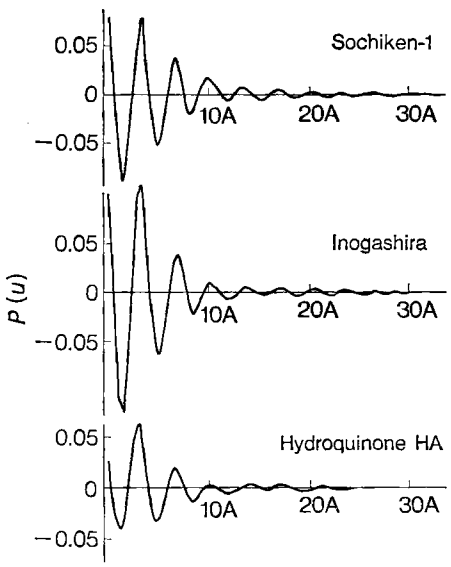


Fig. 10-2. Fourier transform, $P(u)$, of A type and hydroquinone humic acids.

TABLE 10-1
Some parameters of layer stacking in A type and hydroquinone humic acids.

	Interlayer spacing d	Packing number	Average number of layers per stack $\bar{n}$	Weight % of stacks per humic acid P_s
Sochiken-1	3.45	8	2.61	32
Inogashira	3.50	9	2.39	28
Kinshozan-PP	3.48	10	2.48	23
Tenmondai	3.48	9	2.66	28
Hydroquinone HA	3.52	5	2.43	13

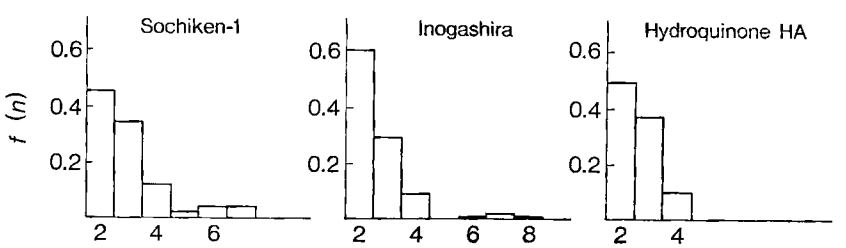


Fig. 10-3. Weight ratio of stacked fraction of n layers per stack, $f(n)$, of A type and hydroquinone humic acids.

tained stacked fractions of two (50 wt%) and three (37 wt%) layers as the main composites of a stack. In addition, a considerable number of layers are supposed to be unassociated, although the portion of single layers could not be calculated by the method adopted in the present study.

The interlayer spacing determined from the diffractograms, d, the average number of layers per stack, $\bar{n}$, and the weight percent of stacks per HA, P_s, are also listed in Table 10-1. The values of $\bar{n}$ ranged from 2.66 to 2.39. The values of P_s for the A type HAs ranged from 23 to 32%, and the one for hydroquinone HA was 13%.

Observation by transmission electron microscope (TEM)

The (002)-lattice image was viewed by the method of Shiraishi (1980) using TEM (Hitachi H500-H, at 100 kV).

Photo 1 is an electron micrograph of Sochiken-1 HA (magnification 280,000×). The (002)-lattice images were clearly observed as a lattice of two to four layers, and enabled estimation of crystallite dimensions of, on the average, about 10Å in the plane of the aromatic layers (L_a). Pollack *et al.* (1971) reported L_a values of about 5 to 10Å.

It is added that the existence of a turbostratic structure by X-ray diffraction analysis and of the (002)-lattice image by TEM was confirmed for A type HAs obtained from Canadian and German Chernozems (Matsui *et al.*, unpublished data).

Based on the experimental results described above, it can be concluded that A type HAs and hydroquinone HA have a turbostratic structure mainly consisting of stacked fractions of two or three layers of an aromatic plane having a diameter of about 10Å.

A type HAs contain large amounts of carbonyl and carboxyl groups as shown in Fig. 9-1, and the ratios of the sums of functional group-C to total-C and functional group-O to total-O were estimated to be 0.52 to 0.72 and 0.85

Photo 1. The (002)-lattice image of Sochiken-1 humic acid. The length of 10 Å is equal to 0.19 cm in the photograph.

to 1.16, respectively (Tsutsuki and Kumada, 1980). These groups as well as nitrogenous constituents, regarded as amorphous parts for X-ray diffraction, might be attached directly or indirectly *via* side chains. The side chains might be composed of saturated and unsaturated aliphatics containing phenolic and quinoid rings, as postulated by Kasatochkin *et al.* (1964).

Judging from X-ray diffraction patterns of A type HA, however, it is probable that the relative amount of side chains is rather small compared to the stacked fractions, because of the absence of the γ-band in the patterns. This is supported by the fact that the sum of weight percent of stacks (P_s) and functional group-C seems to occupy a large part of total-C. That is, the periphery of the stacks is considered to be occupied mostly by the functional groups listed above. In this case, the conjugated double bond systems mentioned in Section 3.3 should be replaced by a turbostratic structure, and the dark brown coloration revealed by A type HA would then be due to the existence of this structure.

For Rp type HAs, the structural model might be composed of large numbers of side chains attached to small and non-stacked layers of aromatic or quinone rings, judging from the lack of the (002)-band. Thus, Rp type HAs seem to be heteropolycondensates, in which conjugated double bond systems exist to some extent. Alternatively, Rp type HAs could be regarded as the products of oxidative disintegration of lignins, probably two or three-dimensionally branched linear polyelectrolytes, to which nitrogenous constituents are incorporated to various degrees. Both models would explain rather weak light absorption in the visible region. Further oxidative disintegration of Rp type HA presumably causes the increase of conjugated C=C double bonds, carbonyl and carboxyl groups and condensed aromatic rings, and consequently the increase of light absorption, resulting in the formation of B type HA.

Comments

(1) As described previously, most HAs obtained from Podzols belonged to P and Rp types, and the exceptional existence of the A type was considered to have originated from charcoal formed by forest fires. Accordingly, the (002)-band found in the HAs of Erica- and Calluna-podzols reported by Pollack *et al.* (1971) would suggest their charcoal origin.

(2) Matsuda and Schnitzer (1972) obtained a high yield of benzenecarboxylic acids from the permanganate oxidation products of methylated Japanese HAs, and stated that these HAs were less humified than O-podzol HA. But the Japanese HAs which they used seem to belong to the A type which, according to the author's concepts of humification, is far more humified than P and Rp types. There may be an alternative interpretation of

the high yield of benzenecarboxylic acids as having resulted from the degradation of condensed aromatic rings constituting the stacked fractions of turbostratic structures.

10.2. Burning as a Possible Mechanism of the Formation of Soil Humus

As stated, A type HAs occasionally found in Podzols are considered to have originated from charcoal formed by forest fire. The fact that A type HA was recovered from charcoal buried at the site supports this idea, but there is a possibility that the A type HA thus recovered originated from the soil A type HA adsorbed on it.

Humus compositions of carbonaceous materials

In order to ascertain that such carbonaceous materials as charcoal and cinder are a possible source of A type HA, humus composition analysis of several such materials was performed and their HAs were compared with soil HAs. This is a model experiment of the lowest level in the forming process of HA.

Samples used in this study were:

No. 1. Charcoal (charred wood) collected from the H and A horizons of a Podzol in the Tyrebagger Forest near Aberdeen, Scotland.
No. 2. Charcoal collected from the A horizon of a Brown forest soil near Tsubame Hot Springs, Niigata Prefecture, Japan.
No. 3. Charcoal collected from the B horizon of a Brown forest soil in the forest of the University of Tokyo, Seto City, Aichi Prefecture, Japan.
No. 4. Cinder (charred wood) collected from the residue of a bonfire.
No. 5. The same as No. 4, but at another place.
No. 6. Commercial charcoal.
No. 7. Homemade cinders (keshizumi in Japanese, soft charcoal created by putting out the fire of burning wood).

After being washed with water, the samples were air-dried, and pulverized in a porcelain mortar. Sample Nos. 6 and 7 which contained no alkali-extractable colored materials were subjected to treatment. Six grams of No. 6 sample was suspended in 150 ml of HNO_3 (1:4) and refluxed for 2 hr, after addition of 3 g lots of $KClO_3$ four times at 15 min intervals. A mixture of No. 7 (0.5 g), $KClO_3$ (1 g), and HNO_3 (1:4, 50 ml) was refluxed for 30 min. After filtration and washing with water, the residues were used for humus composition analysis according to the Nagoya method using a single extraction with 0.1N NaOH.

As shown in Table 10-2, all the samples including Nos. 6 and 7 which

were pretreated with $KClO_3$-HNO_3 contained HAs and FAs. Their amounts, however, varied remarkably. They were larger for sample Nos. 1, 2, and 3, charcoal picked up from the soil, and smaller for sample Nos. 4 and 5, cinders collected from the residue of bonfires. *PQ* values were higher for the former and lower for the latter. Sample Nos. 6 and 7 showed HAs and FAs after $KClO_3$-HNO_3 treatment; this may indicate that the treatment caused the depolymerization and carboxylation of the carbonaceous materials.

TABLE 10-2
Humus composition of carbonaceous materials (d. a. f.).

No.	*a*	*b*	*a*+*b*	*PQ*	$\Delta \log K$	*RF*
1	147	55.4	202	73	0.424	185
2	45.9	22.0	67.9	68	0.484	147
3	419	40.0	459	91	0.666	104
4	6.22	6.22	12.4	50	0.708	57
5	3.42	6.30	9.72	35	0.645	55
6	5.87	4.22	10.1	58	0.631	101
7	69.2	18.6	87.8	79	0.621	113

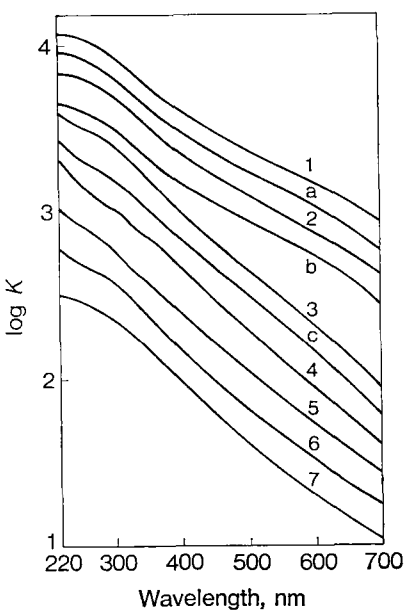


Fig. 10-4. Absorption curves of humic acids. Reference humic acids a and b were obtained from the A_1 and $IIIA_1$ horizons of a Black soil, respectively, and c from a luvisolic Ah horizon: their $\Delta \log K$ values were 0.452, 0.458, and 0.669, and *RF* values 113, 158, and 68, respectively.

$\Delta\log K$ and RF values for HAs of sample Nos. 1, 2, 3, 6, and 7 all corresponded to the criteria for A type ($\Delta\log K<0.7$ and $RF>80$). The shape of their absorption curves (Fig. 10-4) was very similar to that of soil A type HA. On the other hand, HAs obtained from sample Nos. 4 and 5 had higher $\Delta\log K$ values and smaller RF values, and the shape of their absorption curves was similar to that of soil B type HA.

For reference, absorption curves of two A type and one B type HA are also plotted in Fig. 10-4. These were obtained from Black soils in Kyushu and a Luvisol in Canada, respectively. A comparison of the shapes of the curves and $\Delta\log K$ and RF values of these three soil HAs with those obtained from the carbonaceous materials illustrates the close similarity between the two A type HAs and those of sample Nos. 1, 2, 3, 6, and 7. Another close similarity was observed between the B type HA and those of sample Nos. 4 and 5.

It is unlikely that the humus extracted from the samples originated from the soil humus adsorbed on them for the following reasons. (i) The types of HA of soil from which sample Nos. 2 and 3 were collected belonged to P type and Rp type, respectively. (ii) Cinders (Nos. 4 and 5) showed little contamination with soil. (iii) Sample Nos. 6 and 7 were completely free of soil.

Humic acids obtained from the charred residues of plant materials

Following the experiments described above, the plant materials listed in Table 10-3 were respectively placed in a crucible, and charred almost black by a burner. HAs were recovered from the 0.1N NaOH soluble fractions of the charred residue. The optical properties and absorption spectra of HAs dissolved in 0.01N NaOH were determined and are shown in Table 10-3 and Fig. 10-5, respectively (Kumada and Nishio, unpublished data). HA assignations (also in Table 10-3) were made based on the shape of absorption curves and RF and $\Delta\log K$ values.

At the beginning of this section, the author referred to the assumption that carbonaceous materials are a possible source of A type HA. The experi-

TABLE 10-3
Optical properties of humic acids obtained from charred residues.

No.	Material	RF	$\Delta\log K$	HA type
1	White-rotted wood of *Fagus crenata* (Buna)	50	0.671	B
2	White-rotted wood of *Fagus crenata* (Buna)	41	0.659	P_0
3	Brown-rotted wood of *Pinus densiflora* (Akamatsu)	39	0.750	Rp
4	Sawdust of *Cryptomeria japonica* (Sugi)	37	0.671	P_0-L
5	Sawdust of *Chamaecyparis obtusa* (Hinoki)	32	0.704	Rp-L
6	Cellulose (filter paper, Toyo Roshi No. 6)	46	0.726	B

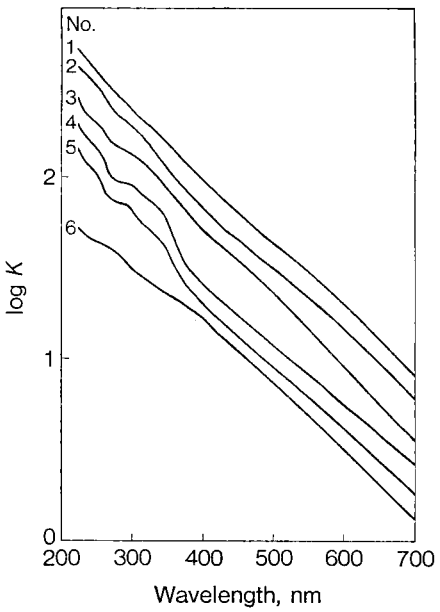


Fig. 10-5. Absorption spectra of humic acids obtained from charred residues.

mental results described indicate that carbonaceous materials and charred products can produce HAs corresponding to various types of soil HA except for P type, and FA too.

Transformation of humic acids by burning

Burning, an indispensable practice in shifting cultivation, causes various changes in the humus composition of soils. To clarify the ecological implications of shifting cultivation, a research project was organized in 1979 (Kyuma, 1983; Oya *et al.*, 1982) in which the author took charge of the soil organic matter studies, and analyzed the changes in pH, carbon and nitrogen contents, nitrogen fertility, and humus composition of the soils from before forest clearing till 30 months after burning (Kumada *et al.*, 1985). Information gathered included the humus composition of the soils sampled immediately after burning which indicated the changes in humus composition by burning.

The experimental site was set up in a forest of Iriomote Island, Okinawa Prefecture. The soil was yellowish, loamy or clay-loamy, acidic with low nutrient status, and assigned to the Dystrochrepts or Yellow soils (Forest Soils Division, 1976).

Trees were felled in about 0.2 ha in the middle of July, 1979 and burnt at the end of August. Immediately after burning, 11 soil samples were taken

TABLE 10-4
Comparison between reburnt spot samples and other samples immediately after burning.[a]

	Layer	9 samples	Reburnt spot	
			a	b
pH	1	5.56±0.43[d]	5.77	5.64
	2	5.09±0.16	5.14	4.74
T-C (%)	1	1.27±0.61	3.04	1.11
	2	1.66±0.52	2.22	1.61
T-N (%)	1	0.149±0.036	0.162	0.075
	2	0.088±0.030	0.107	0.078
C/N	1	19±1	19	15
	2	19±3	20	21
Ce/Ct (%)[b]	1	38.5±3.7	42.8	46.9
	2	38.2±1.8	48.3	33.9
Ch (C mg/g)[b]	1	4.91±1.35	7.14	2.84
	2	2.38±0.81	4.98	2.24
Cf (C mg/g)[b]	1	5.89±1.35	5.88	1.26
	2	4.06±1.01	5.71	3.20
Ch/Cf	1	0.83±0.16	1.21	2.25
	2	0.59±0.13	0.87	0.70
$\Delta \log K$	1	0.617±0.018	0.605	0.531
	2	0.552±0.020	0.556	0.625
K_{600}/c[c]	1	2.80±0.32	4.39	9.88
	2	3.00±0.37	3.47	3.96
Pg (%)	1	0.53±0.14	0.11	0.07
	2	0.87±0.19	0.78	0.23
HA type	1	P_+	B_+	A
	2	P_+	P_+	B_+

[a] Humus was extracted with a mixture of 0.1N NaOH and 0.1 M $Na_4P_2O_7$ at 100°C for 30 min.

[b] Ct: total carbon (mg) per/g soil; Ch and Cf: the amounts (mg) of HA- and FA-carbons per/g soil. Ce=Ch+Cf. Carbon contents were determined by Tatsukawa's method (1966).

[c] c is amount of carbon (mg) in 1 ml of the HA solution. According to Kumada and Ohta (1979), $K_{600}/c \times 15$ is equivalent to *RF*.

[d] Mean and standard deviation.

from depths of from 0 to 5 and 5 to 10 cm, and these were designated as the first and second layers, respectively. Each of the air-dried fine soils was analyzed separately. Fuel sources, such as logs, twigs, and leaves, were in limited supply in this experimental site, therefore fire ran quickly on the soil surface and patches of white ashes, together with half-burnt twigs and logs, remained here and there. The twigs and logs were gathered at several places and reburned. Among 11 soil samples, samples a and b were collected at moderately and intensely burnt spots, respectively. Table 10-4 shows a com-

parison of the average values for nine samples and the reburnt spot samples a and b.

In spot a, the values were not significantly different from the average values of the nine samples, except that in the first layer the Pg content was lower, and the HA present was of the B type. In the first layer of spot b, many distinctive deviations from the average values were recorded: lower carbon and nitrogen contents, smaller Ch and Cf values, lower C/N ratio and Pg content, higher Ch/Cf ratio, and larger K_{600}/c value. The HA belonged to the A type, although that of the nine samples was all of the P type. In the second layer of spot b, a higher $\Delta\log K$ value, lower Pg content and the presence of B type was observed.

Deviations from average values observed in the reburned spot samples were probably due to the degree of burning intensity. The finding that the original P type HA was transformed into B or A type by burning deserves attention, because this suggests that heating of soil by burning resulted in the progress of the humification degree of HA.

Transformation of humic acids by heating of soil

In order to confirm the transformation of Rp or P type into B or A type HA by heating soils, each 70 g of the A horizon soil (<2 mm, moisture content 25%) taken at the forest adjacent to the experiment site was placed in a crucible and heated in an electric furnace at different temperatures and for different periods (Imai, 1983).

The results of the humus composition analysis of the heated and non-heated soil samples are shown in Table 10-5. The higher the temperature, and the longer the duration of heating, the greater was the lowering of carbon

TABLE 10-5
Changes in the humus composition of Iriomote Yellow soil by heating.

Treatment		C (%)	N (%)	C/N	Ce/Ct	Ch	Cf	Ch/Cf	*RF*	$\Delta\log K$	Pg (%)	HA type
Control		3.40	0.192	17.7	40.2	6.24	7.43	0.84	34	0.718	0.34	Rp+
300°C	30 min	3.14	0.188	16.7	40.3	6.33	6.33	1.00	47	0.718	0.14	B±
	60	2.34	0.167	14.0	31.1	5.07	2.21	2.29	97	0.615	0	A
	120	1.65	0.173	9.5	20.9	2.48	0.97	2.56	194	0.610	0	A
400°C	30	2.42	0.150	16.1	24.2	3.70	2.14	1.73	66	0.661	0	B
	60	1.59	0.129	12.3	10.3	0.92	0.72	1.28	158	0.621	0	A
	120	0.56	0.076	7.4								
500°C	30	1.46	0.101	14.5	5.8	0.42	0.42	1.00	176	0.623	0	A
	60	0.83	0.050	16.6								
	120	0.21	0.020	10.5								

(Air-dried soil basis)

and nitrogen contents. The lowering of the former was larger than that of the latter, and the C/N ratio tended to lower with time. The Ce/Ct ratio also lowered. Appreciable amounts of soluble humus were not obtained from the samples heated at 400°C for 120 min and at 500°C for 60 or 120 min. In the other samples, both Ch and Cf values decreased in proportion to the temperature and the duration of heating, but the Ch/Cf ratio rose.

With the intensity of heating, the *RF* value of HA increased, the $\Delta \log K$ value decreased, and the Pg content lowered to zero. HA was transformed from the Rp_+ type to the B_+ type, and further to the A type. These changes in humus composition by heating coincide well with those observed in the soils taken immediately after burning.

The optical properties and absorption spectra of the HAs obtained from an immature Black soil (Ohnobaru) and a buried humic layer (Tsubame, sample #6 in Table 3-2) which were heated at 300°C for 0 to 3 hr, are shown in Table 10-6 and Fig. 10-6, respectively (Kumada and Inoue, unpublished data). The *RF* value of Ohnobaru soil increased with time, and the original B type, very close to the P_0 type, changed into the A type. Contrary to the case of Iriomote soil, however, the $\Delta \log K$ value also increased by heating. Similar changes in $\Delta \log K$ by heating have often been observed, but no interpretation was made. In Tsubame soil, distinct Pg absorption changed little with 1 hr of heating, but had almost and had completely disappeared by 2 and 3 hr of heating, respectively, and A type HAs were obtained.

Based on these experimental results, it is evident that heating of soil having Rp or P type HA yields B or A type HA, *i.e.*, the degree of humification of HA increases by heating the soil. It is not known, however, whether the HA obtained after heating is the transformed product of the original HA or whether it is derived from FA- and/or humin fractions.

TABLE 10-6
Changes in the *RF* and $\Delta \log K$ values of humic acids by heating soils.

Sample	No.	Duration of heating	*RF*	$\Delta \log K$	HA type
Ohnobaru	1	0	46.7	0.672	B
	2	30 min	54.3	0.673	B
	3	1 hr	66.1	0.684	B
	4	2	83.0	0.704	A
	5	3	108	0.736	A
Tsubame	1	0	131	0.347	$A_{\#}$
	2	30 min	141	0.333	$A_{\#}$
	3	1 hr	139	0.424	$A_{\#}$
	4	2	157	0.508	$A_{\pm}$
	5	3	152	0.535	A

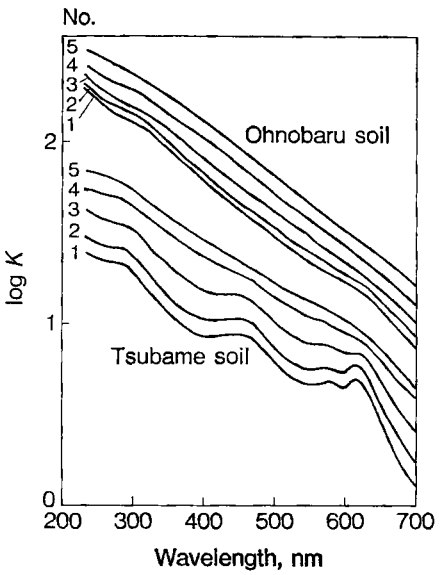


Fig. 10-6. Absorption spectra of humic acids obtained from heated soils.

The burning theory for the formation of humic acid

The above results on carbonaceous materials, charred residue, and soil-heating are summarized as follows: (i) Burning of plant materials can produce HAs very similar to various types of soil HA, except for P type. (ii) Heating, a sort of burning, of soils having Rp or P type HAs can produce B or A type HAs.

These findings confirm that burning must be recognized as one of the mechanisms which causes the formation and transformation of HAs. The author would therefore like to postulate this burning theory as such a mechanism. Here, burning is referred to as a sort of combustion at relatively lower temperature, *e.g.*, 150 to 400°C, accompanied by the carboxylation of burnt residue, and can be regarded as an early stage of carbonization.

It may be premature and unreasonable to conclude, based only on the humus composition analysis (especially optical properties of HAs) that the newly formed or transformed HAs are identical with soil HAs. More detailed studies on other properties as well as their behavior in soils are needed. For instance, it should at least be verified (1) whether the HA existing in the burnt residue can transform into soil HA with respect to existence form, in other words, can form a humus-clay complex or combine with cations in soils, and (2) whether the B or A type HA newly formed by burning soil can

be stabilized and retained in soil. Suitable model experiments will be able to answer these questions.

Harlan's statement (1981) on the use of fire in agriculture is quoted:

"Man had learned to manipulate fire long before any attempt at agriculture. Far back in Paleolithic times man had learned that fire was not only useful for cooking and warmth but could be used to manipulate vegetation and alter the habit of game animals. Fires, deliberately and repeatedly set, tend to reduce woody perennials and open up stands of forest and woodland. Herbaceous vegetation tends to prosper under these conditions and seed collection becomes easier and the yields more abundant. Fires were frequently set in grasslands because grazing animals prefer green shoots that emerge after a burn to new shoots mixed with old, dried residues.

"Surviving hunter-gatherers frequently use fire to alter the vegetation to something more to their liking. . . . The non-agricultural tribes of the Great Basin, USA, actually sowed seeds of wild plants and prepared a seedbed by burning the vegetation in the fall before spring planting.

"Burning vegetation in connection with long-fallow system is especially widespread. In long-fallow systems, a given area of land is established to crops for one to several years and then abandoned for an extensive period ranging from 10 to 25 years. These systems (shifting cultivation) are now most prevalent in tropical regions but were once practiced as far north as Finland."

Thus, burning vegetation, and consequently heating soil, have been practiced by man since ancient times. In addition, forest and bush fires due to various natural reasons have occurred incessantly everywhere in the world. Huge amounts of burned residues must have been produced on the surface and in the interior of soils. It is inferred that these burned residues not only contain original HA and FA, but also can produce HA and FA when they are "weathered" under terrestial soil conditions or subjected to oxidative depolymerization under the influence of oxygen and moisture. These HAs and FAs will be further transformed into lower molecular compounds and finally into inorganic compounds such as CO_2, H_2O, *etc.*. Part of the HAs and FAs might be preserved in soils by complexing with polyvalent cations, sesquioxides, and clay minerals. This is merely speculation, and experimental studies to verify it are necessary.

Problems in the formation of A type humic acid in Black soils

The soils of the Black soil group have thick black or brownish black A horizon, and the black color is due to the existence of A type HA. Although there is still no systematic understanding of the formation of these soils, several researchers have expressed the opinion that the primary source plants for the humus of Black soils are herbaceous vegetation, especially Gramineae such

as *Miscanthus sinensis*, and that burning is essential for the maintenance of grassland (Kawamura, 1950; Kato, 1964; Yamane, 1973; Shoji, 1984).

Under the climatic conditions of Japan, the stable vegetation is forest, and in plateaus, hills, and peneplains where Black soils are widely distributed burning has been practiced deliberately and repeatedly since the dawn of history to hinder the development of shrubs and forests and to maintain grasslands for grazing. The Jōmon people (11,000–3,000 y.B.P.) would have conducted shifting cultivation. Hunter-gatherers who lived from ancient times to the Jōmon Age probably also practiced burning, as Harlan stated.

Burning must have been favorable for the formation of A type HA. This is supported by the finding reported by Shindo *et al.* (1986a,b,c) that HA obtained from charred residue of *Miscanthus sinensis* was very similar to an A type HA extracted from a Black soil with respect to elementary composition, UV, visible, and IR spectra, and X-ray diffractogram. The burning theory, accordingly, may explain the formation of A type HA. However, it should be examined whether the A type HA formed by burning can be transformed into Al-humate.

There are many questions as to the genesis of the humus or HA of Black soils, for instance:

(1) Is it always correct to regard the main source plants of the humus of Black soils as herbaceous and not woody?

(2) Is burning essential for the maintenance of grassland?

Chapter 11 describes that the HAs of buried A horizons of the Black soils younger than 28,000 y.B.P. were all of the A type, except for a few immature exceptions. Were all of these HAs formed under grasslands maintained by repeated burning? It is rather doubtful that the few hunter-gatherers who lived in central and western Japan during the middle and late Würm Ice Age (30,000–11,000 y.B.P.) practiced burning widely, although natural fires would have happened at any time. It may be possible that herbaceous vegetation is maintained without burning or fires as, for example, floor plants under open forests, or permanent grasslands as seen in the alpine zone which have presumably established as a result of the habitat segregation conditioned by soil moisture.

(3) So-called non-volcanogenic Kurobokudo (Andosol) with A type HA is considered to be relict Black soils derived from Akahoya, Aira Tn, and other volcanic ashes (Machida and Arai, 1976, 1978; Arai *et al.*, 1984). Are there any soils derived from parent materials other than volcanic ash and having A type HA, except for Rendzina-like soils? When herbaceous vegetation has been maintained by the burning on parent materials other than volcanic ash and limestone, is Black soil found there?

(4) Assuming that the formation of A type HA and the completion of

its turbostratic structure in Black soils require 200 to 300 years and about 1,000 years (see Chapter 11), respectively, such slow reaction processes might proceed at normal temperature without burning. In that case, even if burning is necessary for the maintenance of grassland or herbaceous vegetation, it may be unnecessary for the formation of A type HA.

(5) Sakai and Kumada (1985b) determined pollen and spore fossils contained in the surface A and buried humic horizons from volcanic ash deposits collected from the archaeological ruins of Shiiji in Numazu city, Shizuoka Prefecture. The geohistorical succession of vegetation taken from a diagram prepared of pollen and spore fossils found in each sample is shown in Fig. 10-7:

Samples No. 14 (*ca.* 26,000 y.B.P.), No. 12 (*ca.* 22,000 y.B.P.), and No. 10 (18,000 y.B.P.): Grasslands predominated in these periods, and consisted mainly of such grasses as *Artemisia*, Gramineae, and Carduoideae. Needle-leaved tree forests composed mostly of *Pinus, Abies*, and *Picea* were also formed.

Samples No. 8 (*ca.* 10,000 y.B.P.) and No. 7 (6,040 y.B.P.): During these periods, grasslands largely composed of Gramineae, *Artemisia*, Car-

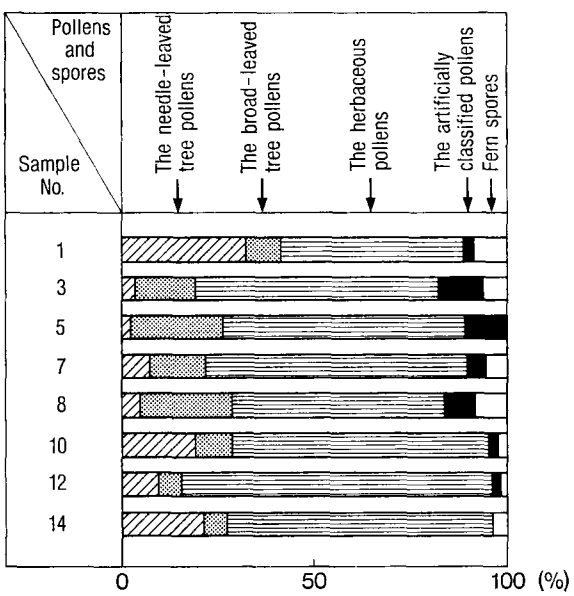


Fig. 10-7. Distribution of needle-leaved tree pollens, broad-leaved tree pollens, herbaceous pollens, artificially classified pollens, and fern spores. The amounts of these pollen and spore fossils were expressed in terms of the percentage of the total pollen and spore fossil grains.

duoideae, and Cruciferae predominated, and broad-leaved tree forests composed of *Castanea* and Lepidobalanus were formed.

Samples No. 5 (*ca.* 4,500 y.B.P.) and No. 3 (*ca.* 3,000 y.B.P.): Grasslands composed mainly of Gramineae predominated. Broad-leaved tree forests were also present as in sample Nos. 8 and 7.

Sample No. 1 (present): Grasslands of Gramineae and *Artemisia* predominated, while needle-leaved tree forests made up of Taxodiaceae and *Pinus* were also dominant. The vegetation types were similar to those presently found.

In summary, throughout the whole period, grasslands holding Gramineae in common predominated, while other herbaceous plants and forests existed at the same time. What original vegetation should we restore on the basis of this study?

Takami and Kubo (1983a) studied two areas in Shizuoka Prefecture where adjoining grasslands and forests exist, and observed Black soils with A type HAs under the former and Brown forest soils with P type HAs under the latter. The parent materials of the soils of each area were volcanogenic deposits, and their mineralogical, physical, and chemical properties were not distinguishable, except for the HA types (Yagi *et al.*, 1983; Tanaka and Kubo, 1983; Tanaka *et al.*, 1983; Yambe *et al.*, 1983; Takami and Kubo, 1983b; Yagi and Kubo, 1983). According to Kubo (private communication), the grasslands had presumably been maintained by burning.

Assuming that the formation of Black soil does not occur without burning by man and/or fires, the soil is a good example of fire being a necessary condition for formation of the great soil group order. Burning can be regarded as one of the soil forming factors. This idea is attractive, but many questions remain.

10.3. Environmental Evidence of the Formation of A Type Humic Acid

Environmental conditions for the formation of A type HA are considered. In addition to the 200 to 300 years which seem necessary for the formation of A type HA in Black soils, Table 10-7 shows the results of analysis on the humus composition of soils taken at the reclaimed polder of Kojima Bay in Okayama Prefecture, Japan (Kumada, 1973b). Following reclamation, the land was used as paddy field for from 3 to 252 years. The pH values of the original polder soils were probably 7 to 8.3 throughout the profile because of the presence of $CaCO_3$ derived from shells. pH values in sample No. 4 ranged from 6.9 to 7.9, with the exception of 6.3 for the first layer (plow layer), suggesting that leaching of Ca ions and acidification have already occurred because of the percolation of irrigation water during rice plant cultivation

TABLE 10-7
Humus composition of reclaimed polder soils.

No.	Depth (cm)	pH	H_E	PQ_1	RF_1	$\Delta\log K_1$	PQ_2	RF_2	$\Delta\log K_2$	PQ_{12}	fHA	fFA	HA type
4-1	0-11	6.3	14.1	59	26	0.804	81	62	0.592	61	86	95	Rp•B
2	11-20	6.9	8.81	56	30	0.776	81	65	0.597	62	68	87	Rp•B
3	20-36	7.3	7.63	48	31	0.773	83	75	0.582	62	47	81	Rp•B
4	36-52	7.1	6.28	53	42	0.697	82	76	0.590	62	57	84	B•B
5	52-62	7.9	6.53	58	53	0.661	78	69	0.587	64	66	84	B•B
6-1	0-13	6.0	16.0	58	43	0.691	81	78	0.548	60	87	95	B•B
2	13-30	6.7	9.36	38	38	0.713	81	81	0.567	53	45	85	Rp•A
3	30-52	6.4	7.95	44	44	0.682	82	78	0.580	57	49	84	B•B
4	52-73	7.1	6.22	50	49	0.652	82	77	0.578	60	57	86	B•B
5	73-83	8.3	5.73	49	50	0.671	85	99	0.545	59	61	90	B•A
7-1	0-11	5.5	15.7	57	37	0.771	63	68	0.555	58	91	93	Rp•B
2	11-33	6.9	4.21	21	28	0.792	81	89	0.579	48	23	83	Rp•A
3	33-72	7.1	4.58	45	46	0.713	86	88	0.570	60	46	86	B•A
4	72-82	8.0	5.15	53	58	0.640	85	85	0.566	64	54	86	B•A
8-1	0-10	4.5	26.5	57	35	0.757	81	60	0.591	58	92	97	Rp•P_0
2	10-13	4.9	18.5	54	34	0.738	80	71	0.570	57	86	95	Rp•B
3	13-16	5.5	5.82	22	21	0.905	74	88	0.593	40	35	84	Rp•A
4	16-40	6.4	3.17	10	31	0.837	75	95	0.596	43	12	77	Rp•A
5	40-77	6.7	5.60	39	28	0.827	85	87	0.580	68	34	79	Rp•A
6	77-87	6.4	7.96	52	57	0.646	93	91	0.548	75	30	85	B•A
9-1	0-12	4.9	22.5	56	34	0.747	74	65	0.562	58	90	95	Rp•P_0
2	12-16	5.1	19.3	51	37	0.732	75	65	0.560	53	87	95	Rp•P_0
3	16-21	5.4	5.53	21	25	0.830	69	104	0.552	34	45	87	Rp•A
4	21-41	6.4	3.20	18	19	0.856	79	117	0.573	52	15	75	Rp•A
5	41-62	7.1	3.89	24	41	0.730	87	112	0.548	64	13	76	B•A
6	62-83	7.1	4.12	57	60	0.644	86	95	0.560	69	46	80	B•A
7	83-93	6.5	3.72	55	57	0.663	84	85	0.581	66	52	83	B•A

The lapse of time after reclamation (years): No. 4: 3; No. 6: 20; No. 7: 60; No. 8: 150; No. 9: 252.

from May to September. The changes in pH values indicate that acidification proceeded not only in the first layer but also throughout entire profiles with the lapse of time. In sample Nos. 8 and 9, the pH values of the first and second layers were about 5 or lower, and those of the fourth to seventh layers ranged from 7.1 to 6.4, where the degree of Ca saturation was presumably near 100%.

Observation of the profiles showed that the original polder soil was in a reductive (anaerobic) condition, and became oxidative with time after reclamation by the diffusion of oxygen during fallowing or the winter crop-growing period. Changes in humus composition following reclamation were

divided into two groups, the upper layers where strong acidification occurred and the underlying layers where acidification was weak.

In the first group, the HA combination type changed with time in the order of Rp•B, B•B, Rp•B, and Rp•P_0, followed by the increase of fHA value from 86 to 92%. In the course of this change, the degree of humification of HAs attained the highest value at sample No. 6-1, and thereafter decreased. The appearance of P_0 type in the $Na_4P_2O_7$-extracted fractions of sample Nos. 8-1 and 9-1 seemed to indicate the retrogression of HA, as was the case for the A horizon of Gray brown podzolic soil in Czechoslovakia.

In the second group, the HA combination type seemed to change in the order of Rp/B•B, B•B, B•A, and Rp/B•A. The fHA values tended to decrease and the RF_{12} value to increase in this order. The HA composition of sample Nos. 9-4 and 9-5 as well as their pH values showed that they correspond to the HA combination type of b(Rp/B•A) described earlier. It is inferred that soil pH of from 7.1 to 6.4, abundant exchangeable Ca ions and oxygen are necessary for the formation of A type HA in the $Na_4P_2O_7$ extractable fraction. These factors are almost identical to those for the formation of A type in Rendzina-like soils, although environmental conditions of polder soils and Rendzina-like soils are seemingly entirely different.

Table 10-7 shows that A type HA was first observed in sample Nos. 6-2 and 6-5. A type HA is supposed to have formed within the 20 years since sample No. 6 was reclaimed. The pH value of sample No. 6-5 whose $Na_4P_2O_7$-extracted HA had *RF* value of 99 was 8.3, distinctly higher than that of No. 6-2 whose $Na_4P_2O_7$-extracted HA had *RF* value of 81. This may indicate that higher soil pH is beneficial to the formation of A type. A type HAs with *RF* values all larger than 100 were found in the layers of sample No. 9, but were not found in layers of younger reclamation ages. These findings suggest that 20 years may be enough for the formation of immature A type, but about 250 years or at least more than 150 years (sample No. 8) are necessary for the formation of well humified A type. It is further presumed that turbostratic structure in HA was partially formed within 20 years after reclamation and well developed after 250 years. Certainly burning is irrelevant to the formation of A type HA in these soils.

10.4. Genesis of Soil Humic Acids

In discussing the genesis of soil HA, the author proposes the carbonization theory as a working hypothesis on the formation and transformation of soil HAs and describes factors leading him to this theory.

(1) An HA sample obtained from a soil is an assembly of fractions whose *RF* and $\Delta \log K$ values are never the same and vary in inverse proportion,

and the *RF* and $\Delta \log K$ values determined for the HA mean averages. Accordingly, even if an HA sample is of, *e.g.*, B type on the basis of its *RF* and $\Delta \log K$ values, its fractions separated by suitable means may be assigned as Rp, B, or A type. This was illustrated in Tables 6-4 and 9-3.

(2) Generally speaking, humification of HA proceeds from Rp type, *via* B type, to A type. The last type is regarded as the ultimate form of soil HAs, but each HA type or combination type exhibits a respectively stable form in each soil from which it is extracted, although in strongly acid soils, Rp type is in reality replaced by P type.

(3) Based on information afforded by X-ray diffraction and TEM, A type is characterized by the existence of a turbostratic structure mainly consisting of stacks of two or three layers of aromatic (hexagonal carbon) plane about 10Å in diameter. The content of carbonyl and carboxyl groups increases in the order of Rp type < B type < A type. Rp and B types are respectively regarded as immature and transitional types of A type.

(4) It is probable that burned products of plants are a source of soil humus, especially A type. As discussed in Section 10.2, however, it is entirely unknown to what extent the HAs derived from burned products participate in soil humus.

(5) Environmental evidence shows that 200 to 300 years are necessary for the formation of A type HA in Black soils, and 150 to 250 years in reclaimed polder soils. While burning might have been practiced in the Black soils, it was not in the polder soils, making it likely that turbostratic structure in HA can form at normal temperature within 150 to 300 years.

(6) The formation of turbostratic structure at normal temperature is supported by existence of the structure in artificial HAs prepared from hydroquinone and catechol in alkaline solution at normal temperature (Pollack *et al.*, 1971; Matsui *et al.*, 1984).

(7) Turbostratic structure implies an early stage of carbonization of organic matter (Ohtani and Sanada, 1980). Since the nature of A type HA is represented by this structure, its formation at normal temperature deserves recognition as the main mechanism of A type formation. The transformation of HA from Rp type, *via* B type, to A type can be regarded as the pathway of carbonization under soil conditions. Accordingly, the author proposes the carbonization theory as a working hypothesis on the genesis of soil HA.

The carbonization theory

This theory is defined as the idea that the formation and transformation of soil HA are an early stage of carbonization under soil condition, that is, at normal temperature and pressure, in the presence of oxygen and moisture, with the participation of soil inorganic and organic constituents as catalysts,

and in fields where biotic activities or detritus food chains of soil organisms occur. Factors such as starting materials, reaction mechanisms, environmental (soil) conditions are considered in order to advance the theory, although most of the discussion is rather speculative.

Starting materials One of the most probable starting materials is lignins. Because of being high molecular polymers with irregular structure, lignins are thought difficult to serve as substrates for microorganisms, but may be transformed into HA by undergoing autoxidative depolymerization involving the formation of various conjugated double bonds and carbonyl and carboxyl groups as well as demethoxylation, hydroxylation and combination with nitrogenous constituents. In the latter stage of this transformation process, the formation of condensed rings consisting of two to four benzene rings, and subsequently the stacking of two to three layers of condensed rings, *i.e.*, the formation of turbostratic structure, occurs gradually. In the course of this transformation process, the beginning, middle and final stages are thought to be represented by Rp, B, and A type HAs, respectively. It seems almost certain that Rp and B types are originated from the autoxidative disintegration of lignins, but there is no evidence which shows that the transformation of lignins can result in A type HA.

Other important sources are presumably phenols and quinones (including polycyclic and extended quinones) originating from plants and microorganisms; they may be transformed into HAs by suffering autoxidative polycondensation. In contrast to lignins, it is at present difficult to guess the HA type originated from these materials and its transformation. But there is a possibility that the materials or their autoxidative polycondensates are stabilized by combining with Ca or Al ions and are transformed into A type HA.

Organic materials participating in the formation of HAs are, of course, not limited to those mentioned. It is reasonable to suppose that humification is a generic term given to extremely complicated formation and transformation processes of brown or dark-colored substances which, in soils where biotic activity is occurring, irrespective of the vital force, proceed autoxidatively in the modes of both polycondensation and depolymerization under the participation of inorganic and organic catalysts (*e.g.*, OH ions, Fe and Mn oxides and oxidases). Humification is in principle an abiotic reaction, and the kinds of materials participating in the formation of the products are immensely diverse. Various dark-colored substances such as melanins, melanoidins, microbial brown pigments are formed in nature, and these may seemingly serve as precursors of soil humus. But their resistance to biological and chemical oxidation differs and the substances which are stable and/or stabilized when combined with inorganic constituents (*e.g.*, Ca and Al ions and clay

minerals) under respective soil conditions may take part in the humification process.

Reaction mechanisms Transformation of HA from Rp type to B type, and further to A type is accompanied by (i) the increase of $RF(E_6^{1\%})$ and decrease of $\Delta \log K$, (ii) the increase of carbon and oxygen contents and O/H ratio and the decrease of H/C ratio, (iii) the increase of carbonyl and carboxyl group contents, *etc.*. In an X-ray diffractogram, Rp, B, and A types are characterized by the γ-band, a composite of the γ- and (002)-bands, and the (002)-band, respectively.

The inference that carbonization in soil is initiated by the development of various conjugated double bond systems, followed by the formation of condensed rings and is completed by the stacking of condensed rings has been deduced on the basis of the information described above. This should be examined critically, but it will surely be found that the carbonization of soil organic matter is a sort of oxidation and proceeds in the mode of radical reaction. Carbonization in soil differs distinctly from carbonization at high temperature in that oxygen takes part in the reaction process and quinone type condensed rings are presumably incorporated in the stacks of turbostratic structure.

Soil conditions For development of the slow series of reactions in the soil which result in turbostratic structure formation, part of the starting organic materials must be preserved throughout the period without suffering from mineralization or leaching. Exchangeable Ca ions in alkaline to weakly acid soils and hydroxyl-Al ions (Wada, 1977) in acid to strongly acid Black soils can be useful in maintaining these organic materials by the formation of Ca- and Al-humus complexes. Catalytic action by hydroxyl ions in alkaline soils, oxidases in weakly acid soils and Fe and Mn oxides in acid to strongly acid soils may accelerate autoxidative changes of organic materials. Furthermore, the ultimate reaction product, A type HA, is sustained for a long time in the form of Ca- or Al humate.

Admittedly, the carbonization theory is at present no more than a working hypothesis. However, it is expected that it will be of use in the systematic understanding of the genesis of HA.

COMMENTS

(1) It was pointed out earlier that A type HAs often found in strongly acid soils where P type HA predominated were supposed to be closely related to the Pg fraction. Apart from this, there is a possibility that the Pg fraction is one of the sources of A type HA, for the following reasons:

(i) As indicated in Fig. 3-6, ΔE_1 spectra of all A type HAs showed Pg

absorption, despite their ordinary absorption spectra not showing it, suggesting that A type potentially contains the Pg fraction or DHPQ moiety. (ii) X-ray diffractograms of P type HAs showed the (002)-band as well as the γ-band, although the intensity of the former was far weaker, suggesting that the (002)-band might be due to the stack of DHPQ planes. (iii) Figure 10-6 showed that the A_{+++} type HA transformed into the A type HA by heating; perhaps, under certain soil conditions, the Pg fraction or its DHPQ moiety is so readily incorporated in the turbostratic structure that Pg absorption cannot be observed in an ordinary absorption spectrum.

(2) The data in Tables 6-1 and 7-3 showed that tannin-like substances disappeared in a very early stage of humification and their contribution to light absorption of HAs also vanished. However, hydrolyzable and nonhydrolyzable tannins (Nishioka, 1986) may participate in the formation of HAs through oxidative polycondensation and oxidative depolymerization, respectively. Behavior of tannins and flavonoids (Burges *et al.*, 1964) during humification requires study.

(3) As shown in Fig. 8-1, changes in absorption spectra of lignins by treatment with 6N HCl were fairly analogous to those of soil HAs with respect to the progress of humification, but the treatment was too drastic and A type-like HA was not obtained. It is recalled that Springer (1938) and Simon and Specichermann (1938) regarded lignin as the main source of HA; model experiments of various levels using lignin as a starting material should be carried out.

(4) B type thus seemed to be transformed into A type HA during the course of humification. In this context, it is noted, however, that Springer (1938) considered gray HA different from brown HAs structurally and genetically.

Chapter 11

Diagenesis of Humus

11.1. Diagenesis of the Humus of Black Soils

Buried humic layers often found in the profiles of Black soils were past A horizons buried by subsequent volcanic fallout. Accordingly, the quantity and quality of the humus of these layers reflect the humus composition of the A horizons at that time and its change with the period since burial. The humus of buried humic layers of a known age affords a good example of the diagenesis of organic matter (Aicken *et al.*, 1985) under terrestrial conditions.

Humus characteristics of the six profiles of Black soil were studied in detail by Ohtsuka (1974a, b, c, 1975), Ohtsuka and Kumada (1974), Yoshida *et al.* (1978, 1979), Yoshida and Kumada (1978, 1979a, b, 1980, 1982), Sakai *et al.* (1982a, b) and Sakai and Kumada (1982, 1985a, b). Of the results, total carbon, C/N ratio and humus composition are described. In those samples whose ^{14}C age was not determined, age was estimated from that of the adjacent upper or under layers; the oldest was 28,000 y.B.P.

The period from 28,000 to 14,000 y.B.P. was part of the Würm Ice Age, and the mean annual temperature was 3 to 5° lower than that at present. The post-glacial age began about 11,000 y.B.P. and the temperature rose gradually, attained maximum at 6,000 y.B.P., 2 to 3°C higher than at present, and then went down. The period from 4,000 to 2,000 y.B.P. was 1 to 2° cooler than the present day.

Carbon content and C/N ratio

Figure 11-1 shows the relations between the total carbon content or C/N ratio of the samples and their ages. The total carbon content of the

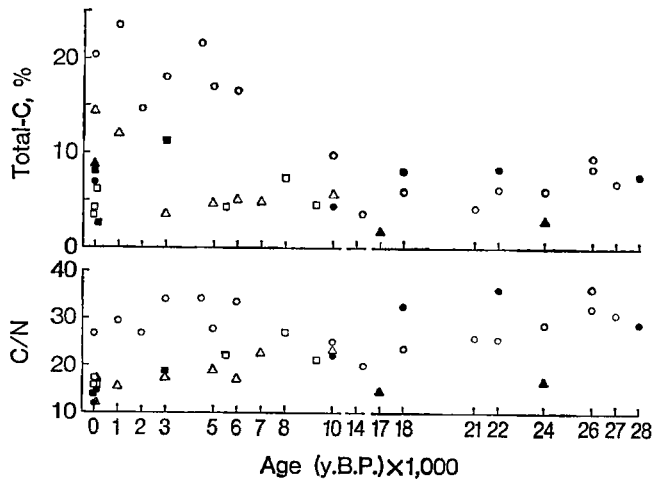


Fig. 11-1. Relationships between total carbon content or C/N ratio and age. □: Ohnobaru; ■: Tarumae; △: Yubunebara; ▲: Mitaka; ○: Shiiji; ●: Ashitaka.

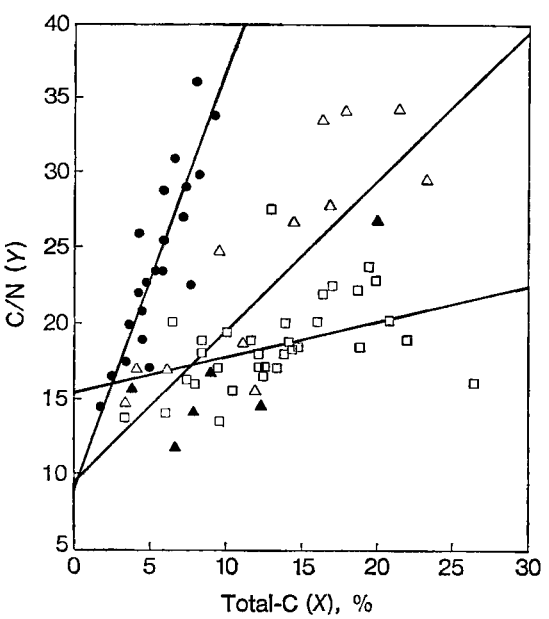


Fig. 11-2. Relationship between C/N ratio and total carbon content. △: group 1, $Y=9.53+0.992X$, γ: 0.792 (99.9%); ▲: present A horizon; ●: group 2, $Y=9.22+2.74X$, γ: 0.908 (99.9%); □: current A horizon samples (Kobo and Oba, 1974), $Y=15.3+0.241X$, γ: 0.420 (98%).

soil samples belonging to the Würm Ice Age and the post-glacial age ranged from 9.4 to 1.7% and 23.3 to 1.6%, respectively; no values of the former exceeded 10%, while more than half those of the latter exceeded 10%. The C/N ratio of all soil samples ranged from 34 to 12 and there was no difference between the two groups. Generally speaking, there seemed no distinct relation between the total carbon content or C/N ratio of soil samples and their ages.

Figure 11-2 shows the relation between total carbon content and C/N ratio of the samples. Again, the samples were divided into two groups: the first was composed of surface soils and soils younger than 5,000 y.B.P. and are distributed widely in the figure. The second group comprised soils older than 5,000 y.B.P., and a highly positive correlation existed between the total carbon content and C/N ratio. Shiiji soil samples younger than 10,000 y.B.P. were included in the first group, and those older than 10,000 y.B.P. in the second group. The present surface soils are shown by black triangles. Current A horizon samples of Black soils with A type HA reported by Kobo and Oba (1974) are also plotted.

Linear regressions and correlation coefficients between total carbon and C/N ratio for both groups and Kobo and Oba's samples are shown. These figures clearly indicate that (i) the range of total carbon contents for the current A horizon samples was very wide and the C/N ratios were relatively low, (ii) with elapsed time after burial, the decomposition of humus proceeded accompanied by the increase in C/N ratio, which tended to be higher in proportion to the amount of humus, and (iii), finally, a highly significant correlation was established between total carbon content and C/N ratio of the residual humus after a certain time has passed. Changes in the relation between total carbon content and C/N ratio of the soils with time after burial seem to reflect one aspect of the diagenesis of humus in Black soils.

Total carbon content seems to vary with respective profiles, probably depending on environmental conditions (Fig. 11-1); on the other hand, the decomposition of humus after burial appears to take place depending on the amount accumulated rather than environmental conditions, resulting in the establishment of a highly significant correlation between total carbon and C/N ratio of residual humus. The mechanism of the transformation, however, should be investigated in future.

Humus composition

The relations between the H_E, PQ_{12}, fHA, fFA, RF_1, and RF_2 values of the samples and their ages are shown in Fig. 11-3. The highest H_E value of a sample from the Würm Ice Age was 105 ml (0.1 N $KMnO_4$ per g), while

it was 400 ml for samples of the post-glacial age. The PQ_{12} values were high, ranging from 58 to 87%.

As shown, fHA and fFA values lowered with time, but the rates of lowering were diverse with respective profiles and also with HA and FA. This lowering of fHA and fFA values with time may be related to the transformation of aluminum combining with humus from alkali-soluble form to pyrophosphate-soluble form, *i.e.*, presumably from non-crystalline to crystalline. All HAs of the buried layers were A type. Both RF_1 and RF_2 values tended to increase during the post-glacial age, then decrease toward the Würm Ice Age, and remained nearly constant till 28,000 y.B.P.

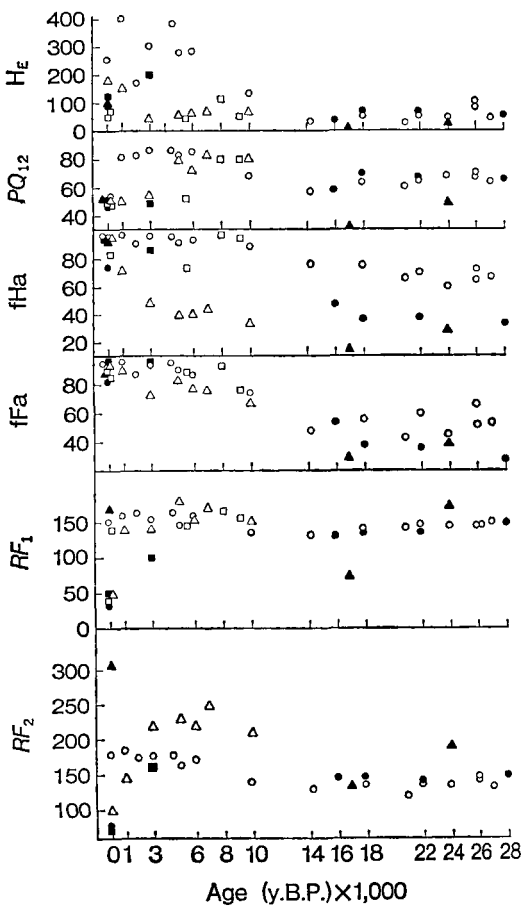


Fig. 11-3. Relationships between the humus composition and age of the present A and buried humic horizons of Black soils. □: Ohnobaru; △: Yubunebara; ○: Shiiji; ■: Tarumae; ▲: Mitaka; ●: Ashitaka.

Generally speaking, the changes in humus composition of humic layers with the lapse of time after burial seemed characterized by the lowering of fHA and fFA values and the increase followed by the decrease of *RF* values. However, these changes varied remarkably with respective profiles. The amount of humus remaining in the buried layers also varied from profile to profile, although PQ_{12} values tended to be generally very high compared to those of present A horizons.

Properties of humic acids

Recently, Matsui *et al.* (1986) prepared HA samples from the soils of present and buried A horizons of certain Black soil profiles using the Nagoya method with 0.1 N NaOH as extractant, and determined their elementary composition, optical properties, and X-ray diffraction.

Samples:

Hayakida: Hy 1; A, formed on the Ta-a (1739 A.D.), Hy 2; IIAC, between the Ta-b (1669 A.D.) and Ta-a, Hy 3; IIIA, between Ta-c (1640* y.B.P.) and Ta-b, Hy 4; IVA (8,940* y.B.P.). The Ta-a, b, c are volcanic fallout from Mt. Tarumae.

Kanoya: Ka 1; A_{11}, Ka 2; A_{12}, Ka 3; IIA (1,000 y.B.P.), Ka 4; IIIA (3,000 y.B.P.), Ka 5; IVA (4,000 y.B.P.), formed on Akahoya volcanic ash (6,400* y.B.P.).

Yubunebara: Yu 2; IIA (864–800 A.D.), Yu 3; IVA (3,500 y.B.P.), Yu 4; VIA (7,000* y.B.P.), Yu 5; VIIA (10,000 y.B.P.).

Shiiji: Si 1; A_{11}, Si 2; A_{12}, Si 3; IIA_{11} (3,000 y.B.P.), Si 4; IIA_{12} (4,500* y.B.P.), Si 5; III/IVA (6,040* y.B.P.), Si 6; IVA (10,000 y.B.P.), Si 7; VIA (18,000* y.B.P.), Si 8; VIIA (22,000 y.B.P.), Si 9; VIIIA (26,000 y.B.P.).

Note: The y.B.P. values with * were determined by the ^{14}C method. Others were estimated and are therefore not reliable.

Humic acids of present A horizons Five out of 22 HA samples were obtained from present A (A_{11}, A_{12}) horizons, and their $E_6^{1\%}$ values ranged from 22 to 120. Since HA transforms from Rp type to P_0 type, B type, and A type, successively, with the maturing of Black soils, it is considered that a series of HAs on the basis of $E_6^{1\%}$ value, *i.e.*, Hy1—Ka 1—Ka 2—Si 1—Si 2, indicates the maturing of HA in the present A horizon of Black soils. Accordingly, changes in the above stated properties are first examined for the series of HAs.

The $E_6^{1\%}$ values of HAs seen in Fig. 11-4 show that an increase of $E_6^{1\%}$ value was accompanied by a lowering of E_4/E_6 ratio. As for elementary composition, with increasing $E_6^{1\%}$ value, C% increased throughout the series of HAs, but H%, N%, H/C and N/C ratios increased from sample

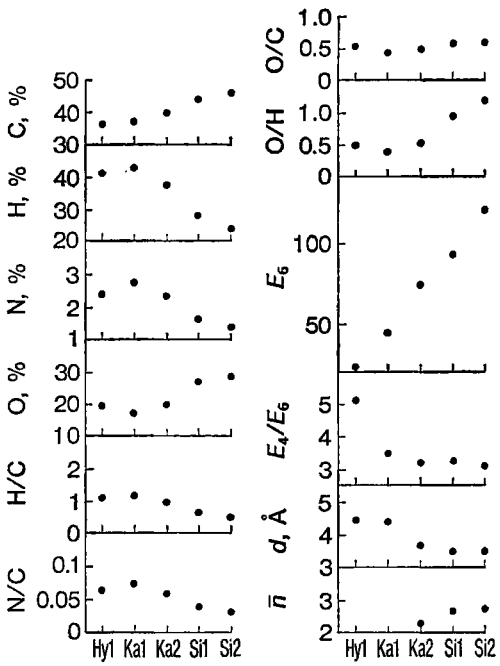


Fig. 11-4. Parameters of the elementary composition, optical properties, and X-ray diffraction of humic acids obtained from the present A horizons of Black soils.

Hy 1 to Ka 1, then decreased in the following members, and the reverse relation was observed for O% and O/H ratio. O/C ratio remained almost unchanged. Changes in elementary composition from sample Hy 1 to Ka 1 may be due to the enrichment of nitrogen in HA molecules in the early stage of humification, as suggested by Kuwatsuka *et al.* (1978).

X-ray diffractograms illustrated in Fig. 11-5 show that HA samples are divided into three groups, *i.e.*, those having γ-band, γ- and (002)-bands, and (002)-band, and respectively called groups 1, 2, and 3. Samples Hy 1 and Ka 1, Ka 2, and Si 1 and Si 2 belonged to groups 1, 2 and 3, respectively. As seen in Fig. 11-4, d (interlayer spacing) values decreased and $\bar{n}$ (average number of layers per stack) values increased for the last three members in the order of HA series.

The maturing process of HAs in Black soils thus is characterized by the increase of light absorbability, the increase of carbon and oxygen contents, the decrease of hydrogen and nitrogen contents, and the development of a turbostratic structure. These findings serve to illustrate the carbonization theory of the formation process of HA discussed in Section 10.4.

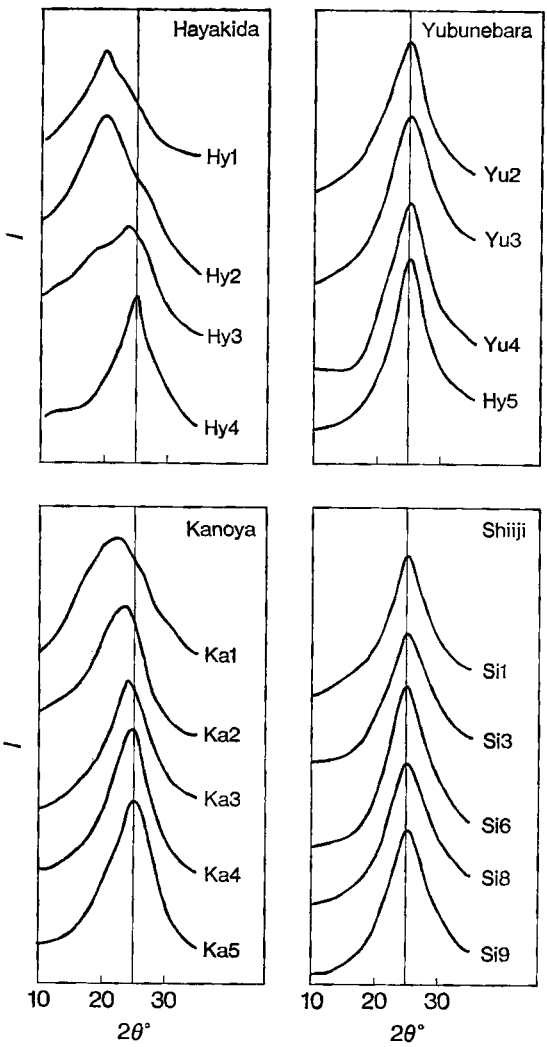


Fig. 11-5. X-ray diffraction profiles of humic acids obtained from present A and buried humic horizons of Black soils.

Humic acids of buried humic horizons Properties of HA samples against ages are plotted in Fig. 11-6. Changes in properties of HAs with the lapse of time after burial by volcanic fallout were very similar to those of the maturing process of present A horizons. Generally speaking, changes in elementary composition such as the increase of C%, O%, and O/H ratio and in optical properties seem to occur abruptly within about 1,000 years

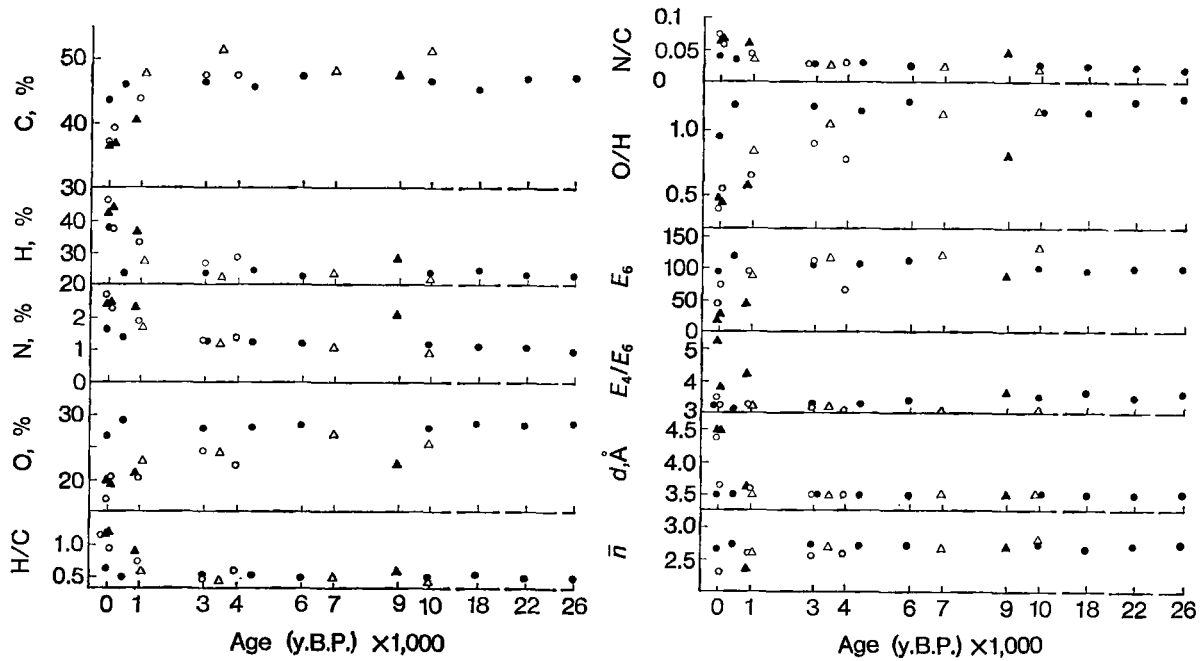


Fig. 11-6. Relationships between various properties and age of humic acids obtained from the present and buried A horizons of Black soils. ▲: Hayakida; ○: Kanoya; △: Yubunebara; ●: Shiiji.

after burial, almost cease after 3,000 years, and are thereafter minimal. Other points noted were: (i) Judging from the changes in elementary composition and optical properties, the transformation of HAs in Hayakida and Kanoya profiles seemed slower than in Shiiji and Yubunebara. As Hayakida soils were taken in Hokkaido, the slower changes were probably related to the lower temperature of the sampling site. Since the Kanoya profile had developed on an impermeable Akahoya ash layer, poor drainage or humid conditions may have obstructed the progress of humification. (ii) $E_6^{1\%}$ values of samples Si 7, 8, and 9 belonging to the Würm Ice Age tended to be smaller and E_4/E_6 ratios higher than those of the RII period. This may imply either the delay of humification due to the cool temperature or the degradation of HAs as a result of diagenesis.

According to X-ray diffraction analysis, sample Hy 2 belonged to group 1, Hy 3 to group 2, and the rest to group 3. Since sample Yu 2 (800–864 A.D.) belonged to group 3, the formation of turbostratic structure seems to have been completed within about 1,000 years after burial, as was also true of the abrupt changes in elementary composition and optical properties. Alternatively, it may be said that these reactions are almost completed within 1,000 years after soil formation begins and irrespective of burial. In this context, 200 to 300 years seem necessary for the formation of A type HA in Black soils.

The diagenesis of HAs after burial is characterized by changes in elementary composition, optical properties, and X-ray diffraction which are similar to the maturing process in the present A horizon. This means that diagenesis is, in principle, the same as maturation and can be understood, since both processes are only carbonization in terrestrial soil conditions. Most HAs of buried A horizons, however, are distinguishable from those of present A horizons as shown in Figs. 5-3, 5-5, and 5-6. As a matter of fact, conspicuous differences between HAs of present and past A horizons can be expected because they have experienced differences in such factors as age, a continuous supply of fresh plant remains, *etc.*.

Summary

Diagenesis or changes in the humus of Black soils with the lapse of time after burial are summarized as follows:

1. The amounts of humus in the present A horizons varied considerably among soils and the C/N ratios were comparatively low. In soil samples following a certain period (about 5,000 years for 3 profiles and 10,000 years for 1 profile), highly significant correlation existed between the amount of total carbon and C/N ratio.

2. In general, fHA and fFA values decreased with time, but the rates of decrease varied remarkably with respective profiles. *RF* values tended to increase at first, then decrease, and attain equilibrium during the Würm Ice Age; PQ_{12} values tended to be higher than those of present A horizons.

3. Diagenesis of HAs of buried A horizons were characterized by increased light absorption, increased carbon and oxygen content, decreased hydrogen and nitrogen contents, and the development of a turbostratic structure. Similar changes were suspected to occur in HAs of the present A horizon with the passage of time even without burial by volcanic fallout.

11.2. Elementary Composition and Optical Properties of Sedimentary Humic Acids and Fulvic Acids

Although the comparative humus chemistry mentioned in Chapter 1 is beyond the scope of this book, the elementary composition and $E_6^{1\%}$ and $\Delta\log K$ values of sedimentary HAs and FAs are illustrated in Table 11-1 for reference. Lake sedimentary HAs and FAs in Japan were obtained from core samples of surface muds, except for those of Lake Haruna (1), the deepest core sample of which ranged from 20 to 24 cm with an age of about 1,000 y.B.P. (Ishiwatari, 1972). The age of the deepest core sample from the Sea of Japan was about 70,000 y.B.P. (Ishiwatari, 1972). All the data listed in Table 11-1 were cited from a handout delivered by Professor Ishiwatari at his lecture "Geochemistry of Sedimentary Humic Substances" held at Nagoya University, September 1, 1984.

As seen in Fig. 11-7, HAs were distributed widely from the B area to FA area in the C′-H′-O′ diagram. Among HAs of Japan, those locating in the HA band had carbon (C′) contents ranging from 34.86 to 39.09%, and were discriminated from the rest of the 31.62 to 33.88% located on the FA

TABLE 11-1
E_6 and $\Delta\log K$ values of sedimentary humic acids (Ishiwatari, 1984).

Sample		$E_6^{1\%}$	$\Delta\log K$	Sample		$E_6^{1\%}$	$\Delta\log K$
L. Haruna	0-8 cm	13.4	0.68	L. Shirouma-oike		15	0.69
(1)	8-12	11.9	0.76	L. Shoji		12	0.61
	12-16	14.5	0.69	L. Aoki		23	0.67
	16-20	13.0	0.78	L. Kizaki		29	0.66
	20-24	13.4	0.74	L. Haruna (2)		13	0.64
L. Motosu		10	0.71	L. Nakatsuna		33	0.65
L. Nishinoumi		14	0.69	The Sea of	5-10 cm	14	0.80
L. Kawaguchi		16	0.71	Japan	20-30	11	0.78
L. Yamanaka		13	0.66		80-90	18	0.67
L. Tsuruga-ike		13	0.65		90-100	19	0.68

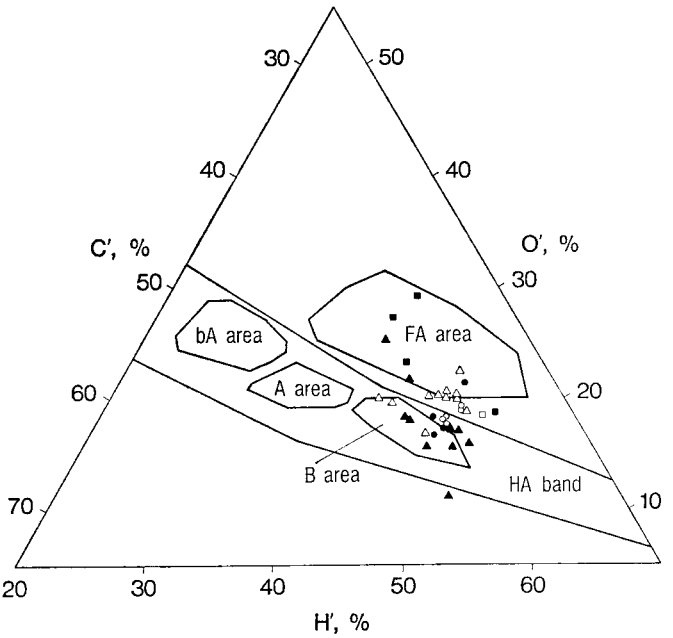


Fig. 11-7. C'-H'-O' diagram of some sedimentary humic acids and fulvic acids (data from Ishiwatari, 1984). ○: HA, L. Haruna (1); ●: HA, the Sea of Japan; △: HA, the rest of Japan; ▲: HA, foreign countries; □: FA, Japan; ■: FA, foreign countries.

area side. $E_6^{1\%}$ values of the former and latter were 13 to 33 and 10 to 16, respectively. No difference between the two was seen in $\Delta\log K$ values. A weak shoulder-like absorption near 405 nm in the absorption spectra presumably due to the pheopigments may have disturbed the establishment of a reciprocal relationship between $E_6^{1\%}$ and $\Delta\log K$. In foreign HAs, 9 out of 11 samples were located on the HA band and the rest in the FA area. Their carbon (C') contents were all in the range of 34.13 to 38.18%.

Sedimentary HAs may be said to correspond to soil Rp type HA or FA, and those corresponding to the former might have been partly originated from soil HA. All the FA samples were distributed in or near the FA area.

Among 5 HA samples from Lake Haruna (1), the younger 2 and the older 3 were respectively located in the FA and B areas, and in the case of the HA samples of the Sea of Japan, the second youngest one belonged to the FA area, and the rest were located in the B area. The scope of distribution of these samples obtained from the same sites was rather restricted in comparison with the wide distribution of samples obtained from different lakes. This suggests that the diagenesis of lake sedimentary HAs during

1,000 years and sea bottom HAs during 70,000 years have undergone no remarkable changes in elementary composition and optical properties. The data cited above, however, were too few to draw any definite conclusions on the diagenesis of sedimentary HAs and FAs.

Comment

According to Ishiwatari (1985), humic and fulvic acids in lake sediments are probably melanoidin-like materials. This suggests that melanoidin-like substances are stable under lake sedimentary conditions. On the contrary, it is considered that melanoidins are unstable and readily decomposed under terrestrial soil conditions, as is true of chlorophyll.

Chapter 12

Complementary Remarks

In Chapter 1, the author mentioned why he had decided to avoid using the term "humic substances" in this book. At the same time, however, he stressed the necessity to define this important concept in terms of chemistry. As discussed earlier, humic substances are defined as the chemical configuration responsible for visible light absorption in HA. They imply a series of transitional configurations initiated by the formation of conjugated double bond systems randomly distributed in linear polymers, and ending with the formation of turbostratic structure. The term humification is referred to as the formation process of the configurations accompanied by the increase of light absorption and carbonyl and carboxyl groups; humification can be regarded as an early stage of carbonization in soil. It is inferred that the degree of humification of humic substances depends on soil conditions, with soil pH being one of the decisive factors; the higher the soil pH, the higher the degree of humification, except in Black soils. Grouping of HAs into Rp, B, and A types is actually the grouping of humic substances on the basis of the humification degree which increases in this order.

Perhaps some remarks are appropriate here on the relation between humic- and non-humic substances and methodology for humus chemistry.

Humic- and non-humic substances

If the above idea on humic substances and humification is permissible, it may be reasonable to consider that humic substances are formed not only *from* but also *in* non-humic substances, indicating that the former cannot be separated from the latter. In other words, it is not rational to think that humus (II on p. 2) is divided into humic substances (IIa) and non-humic substances (IIb), and IIa into HA, FA, and humin. We must realize that

HA, FA, and humin are respectively composed of IIa and IIb, or humified and non-humified parts, and it is theoretically as well as practically impossible to strictly separate them from each other. Although it is possible that some kinds of organic substances exist which are entirely unhumified, *i.e.*, have not been structurally altered by oxidation, it seems important to establish suitable experimental criteria for the judgment of humification.

As shown in Chapter 9, the main components of acid-hydrolysates of HAs are amino acids, phenolic substances, sugars and uronic acid, and the sum of these substances is 6 to 35% of total carbon. The amount of acid-hydrolyzable organic matter may serve as an index of the level of non-humic substances, but the following should be taken into consideration as factors of underestimation. (i) Acid non-hydrolyzable organic matter, *e.g.*, lignin and non-hydrolyzable tannin cannot be estimated. (ii) The formation of artificial humic substances during acid-hydrolysis may not be neglected. Nevertheless, it may be said that the level of non-humic substances decreases with the progress of humification.

FA is also considered to be mainly composed of phenolic substances, polysaccharides, and proteinaceous constituents. Clear-cut separation of these groups is hardly possible because they combine to varying extents through covalent, ionic, and hydrogen bonds.

According to Lowe (1975, 1980), Ca/Cf ratio (the proportion of fulvic carbon recovered in the polyphenolic fraction) varied from 18 to 68%, and was related to horizon types. The PVP- and XAD-adsorbed polyphenolic fractions may be generally regarded as humic substances, and the non-adsorbed fraction as a non-humic substance. As with HA, however, there is no guarantee that the non-humic substances are entirely unhumified.

For the development of the chemistry of soil organic matter (SOM)

Sources of SOM are products of living things, *i.e.*, organic substances synthesized by organisms. The chemistry of these organic substances, biological chemistry, is now highly developed. If SOM is merely an assembly of organic substances, the chemistry of SOM should not, in principle, be different from biological chemistry: it is the chemistry of non-humic substances.

We know that SOM and its fractions are composed of humic and non-humic substances. As postulated by Kononova (1966), humic substances are "substances which, because of the peculiarity of their nature, cannot be related to any of the existing groups of organic (biological) chemistry . . . these substances are justifiably included in a separate group termed humic substances." The "peculiarity of their nature" can be attributed to the fact that they are amorphous, brown-colored substances, because such amorphous substances are beyond the scope of organic chemistry. It should be noticed,

however, that the amorphousness of humic substances does not mean that they are really chaotic and disordered. To the contrary, regularities in the elementary composition and optical and other properties of HAs suggest that humic substances are well-ordered, as summarized at the beginning of this chapter.

The classification system of HA adopted in this book is no more than that of humic substances, and in order to deepen our understanding of these substances chemical configurations directly related to the light absorbability of various types of HA and to the increase of their light absorption with the progress of humification should be elucidated. As discussed by Maximov *et al.* (1977), our knowledge of the chemical configuration of HAs and FAs is still very poor, despite efforts by many researchers and progress in instrumental analysis.

Hayes and Swift (1981) predicted some of the types of primary structures which compose humic materials: "It is clear that humic materials contain single-ring aromatic structures, many of which are heavily substituted with aliphatic hydrocarbon, carbonyl, methoxyl, hydroxyl, carboxyl, and other side groups. Some of the aliphatic hydrocarbon structures are likely to be unsaturated; some of these may even have a degree of conjugation, and many are likely to contain various functional groups, including carboxyl. All of these need not necessarily be substituents of aromatic structures. There is no evidence which could exclude heterocyclic, aliphatic, and aromatic structures containing oxygen and nitrogen." This statement seems useful in explaining the chemical configuration of Rp type HA, although the presentation of structural models for conjugated double bond systems as well as linear polyelectrolytes is desirable.

Turbostratic structure, on the other hand, demands that suitable degradation experiments yield considerable amounts of polycyclic rings. This implies that degradation experiments should be projected which premise the existence of polycyclic ring structures; for instance, the dichromate oxidation method explored by Hayatsu *et al.* (1978) for the study of coal structure may be helpful.

It is almost certain that not only P type but also A type HA and FAs contain a perylenequinone nucleus. Biosynthesis of this nucleus is presumably accompanied by the formation of polymers containing a naphthalene nucleus (Allport and Bu'Lock, 1958). Therefore, it seems somewhat curious that various degradation experiments conducted hitherto have been unsuccessful in showing the existence of perylene or naphthalene nuclei, except for those using the Zn dust fusion or distillation method (Sato and Kumada, 1967; Cheshire *et al.*, 1967; Hansen and Schnitzer, 1969; Kumada and Matsui, 1970) and the enzymatic degradation method for naphthalene (Mathur, 1971).

These findings suggest that, in order to establish the chemical configurations characteristic of respective types of HA, degradation experiments suitable to the chemical configurations supposed for each type should be applied.

Studies of the chemical configurations of various types of HA focusing on the mechanisms of light absorption should be one of the main subjects of humus chemistry, and will provide the answer to what humic substances are. A successful study will necessitate the utilization of various experimental techniques in the field of lignin, coal, carbonaceous materials, and organic semiconductors. At the same time, it is important to study carefully and repeatedly the many excellent achievements in humus chemistry produced by our predecessors.

The following sentence from Dr. W. Steelink's review (1963) entitled "What is humic acid?" seems an apt finale.

"Out of sheer cussedness, there will always be a few hardy souls who will want to know the fundamental reason for Nature's wide distribution of this substance, and the method by which she synthesized it."

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